Roger G. Lentle
Patrick W.M. Janssen

The Physical Processes of Digestion

Springer

The Physical Processes of Digestion

Roger G. Lentle • Patrick W. M. Janssen

The Physical Processes of Digestion

Springer

Roger G. Lentle
Institute of Food Nutrition
and Human Health
Massey University
PO Box 11-222, Palmerston North
New Zealand
r.g.lentle@massey.ac.nz

Dr. Patrick W. M. Janssen
Institute of Food Nutrition
and Human Health
Massey University
PO Box 11-222, Palmerston North
New Zealand
p.janssen@massey.ac.nz

ISBN 978-1-4419-9448-6 e-ISBN 978-1-4419-9449-3
DOI 10.1007/978-1-4419-9449-3
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2011927937

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

The authors wish to thank Cheryl and Inge for their love and support.

Foreword

This book is the first comprehensive review of what is known regarding the physical processes of digestion in man and other simple-stomached vertebrates. The authors consider the physical constraints that govern the dissolution and digestion of food particles and the physical properties of whole digesta that influence flow and mixing, and hence the physical process of diffusion of nutrients to and through the gut wall. They move on to examine the effects of the various forms of contractile activity in facilitating these processes in the various segments of the gut. Finally, they consider the actions of the mucinous and cellular components of the gut wall in limiting the permeation and absorption of nutrients and other pharmacological and microbial products. In doing this, they have brought together material from a diverse range of peer-reviewed publications ranging from rheology to mathematical modelling. The work is thorough and authoritative, citing over a thousand peer-reviewed references.

The work will be useful to all who seek to gain a greater understanding of digestive processes, notably those such as food technologists, pharmacologists, physiologists and nutritionists whose work requires the development of strategies to enhance or impair digestive or absorptive efficiency. It will be particularly useful for those who are specialised in particular disciplines who wish to gain a broader overview that is couched in understandable terms. The authors clearly explain necessary physical concepts without recourse to undue mathematical complexity.

The work is most timely given the recent upsurge in public interest in healthy eating, functional foods and nutraceuticals. It provides a sharp scientific focus on understanding the dynamics of nutrient release and uptake, especially in the context of the metabolic syndrome. It is also timely in regard to the exponential increase in the number and diversity of publications within the field. It is thought provoking, with the authors highlighting a number of areas in which the explanations of particular phenomena are equivocal. It is hoped that their clear enunciation of the relevance and importance of these various dichotomies may spur further research interest in these areas.

The authors are to be commended for their efforts and for the fresh insights that they have provided on what is a most fascinating subject.

Paul J. Moughan
Director of the Riddet Institute

Contents

Chapter 1
Introduction

Contents

1.1 Scope

The purpose of this book is to provide an overview of the current state of knowledge regarding the physical processing of food and nutrients within the gastrointestinal tract. The book is aimed at physiologists, pharmacologists, nutritionists and food technologists whose work requires an understanding of the physical processes of digestion. A broad interdisciplinary view is given with careful explanation of any specialist concepts notably in biophysics and rheology.

The scope of the book is necessarily broad. The process of nutrient absorption may be influenced by factors that govern the dissolution of nutrients from the solid phase of digesta, the transit of the nutrients to their site of absorption at the intestinal wall, and the transit of nutrients through the apical membranes of the lining cells, i.e. the enterocytes (Goodacre and Murray 1981). These factors are variously influenced by the physical properties of the particulate and liquid components of digesta, the morphological and molecular characteristics of the various components of the wall of the gastrointestinal tract, as well as the timing (Amidon et al. 1995) and form (Lentle et al. 2007) of its contractile activity.

The book comprises two broad sections and contains a number of chapters written by guest authors who are recognised experts in their field. The first section examines the general factors affecting the digestion and physical disruption of heterogeneous particles followed by specific discussion in respect of carbohydrate (authored by Allan Hardacre and John Monro), protein (authored by Allan Hardacre)

R. G. Lentle, P. W. M. Janssen, *The Physical Processes of Digestion*,
DOI 10.1007/978-1-4419-9449-3_1, © Springer Science+Business Media, LLC 2011

and fat (authored by Matt Golding and Tim Wooster) components of the particles. The section concludes with an examination of the physical behaviour of particulate suspensions and other semi-solid materials of similar structure to digesta within the various segments of the gastrointestinal tract. The second section examines the biological factors that influence mixing and absorption of nutrients, including the effects of propulsive and mixing movements of the walls of the various segments of the gut, and the effects of muscular tension (i.e. tone) in these elements. Finally, the effects of the various layers of the mucosa, including those physical components that constitute the ‘unstirred water layer’, on the process of absorption and digestion are considered.

1.2 The Dynamics of Food Digestion and Absorption

It has become increasingly apparent that the physical processes that govern the digestion of food and the transit of the resultant substances to and through the wall of the digestive tract have a significant effect on the rate and efficiency of their absorption (Goodacre and Murray 1981). These various effects have stimulated the interest of food technologists and pharmacists as they offer a means of modulating such absorption. Particular examples include work demonstrating the influence of the physical form of carbohydrates (Lehmann and Robin 2007) and proteins (Chen et al. 2006), and the cellular organisation of natural foods (Mandalari et al. 2008) on absorption. Similarly, a number of studies have demonstrated the significant influence of viscogenic agents (Dikeman and Fahey 2006) and of the components of the mucus layer (Cone 1999) on flow, mixing and mass transfer within and from the lumen.

The process of digestion comprises a number of interdependent rate-limited processes which culminate in the absorption of nutrients. These processes include fragmentation, solution, enzymatic breakdown and mass transfer, all of which have characteristic time constants or rate kinetics. For example, the digestion of vegetable material and the absorption of contained nutrients may require comminution and trituration to reduce particle size appropriately and to disrupt any impermeable coating (Lucas 2004). The heterogeneous particles must then undergo permeation by digestive secretions to weaken the cellular structure and convert contained nutrients into more readily transferred forms. The dissolved or dispersed nutrients must diffuse from the surface or interior of the particles into the surrounding bulk liquid phase (Kong and Singh 2009). The nutrients must then traverse the lumen to the mucous layer by appropriate mixing of the lumen contents. Finally, they must traverse the mucous layer by a combination of diffusion and convection (solvent drag), the latter resulting from the net absorption of water by the mucosa (Pappenheimer 2001). On arrival at the apical surface of the enterocyte, the nutrients may require further degradation by enzymes liberated from the brush border before being absorbed. The dynamics of that absorption vary with the nature of the nutrient, i.e. whether they undergo passive or active absorption and whether they are absorbed via the paracellular or transcellular route.

Most of these processes occur sequentially, and hence, it is likely that the overall rate of nutrient uptake for a particular combination of nutrient and food matrix will be determined by the slowest of these processes. The digestibility of a food will be influenced by the time scale of the rate-determining process in relation to the time that the food matrix is retained in the segment in which digestion occurs. Thus in a case where the rate of digestion of a food particle is limited by intact cell walls or a surrounding matrix, then the time for digestive secretions to penetrate to the contained nutrient and for it to undergo digestion or dissolution may exceed the time the particle spends in a gut segment that is capable of absorbing it.

Current methods for determining rates of mucosal absorption do not allow the effects of all constituent processes to be evaluated. Experimental limitations can result in the combination of several constituent processes being inadvertently determined, effectively preventing identification of the principal rate-determining process. For instance, the resistance of the mucous layer to the absorption of soluble marker substances was overestimated in early work as it was confounded with the resistance to transit of the marker across the lumen (Levitt et al. 1990). Similarly, it is difficult to separate the resistance to transit through the mucous layer from the resistance to transit into the intestinal epithelium (Fagerholm and Lennernäs 1995).

The dynamics of some of these processes are likely to be non-linear as physical or physiological limitations may constitute upper bounds to properties or flow rates. Thus the efficient absorption of a nutrient that can readily transit the gut lumen and wall but is sparingly soluble may require a larger volume of liquid to entirely dissolve it than can be secreted by that segment. Similarly, active transport mechanisms mediating the transcellular absorption of a readily soluble and digestible substance may become saturated so that transit by passive absorption must occur above a certain threshold concentration (Reigner et al. 1990).

1.3 Mixing and Other Effects of Contraction of the Gut Wall

Contractile activity in the wall of the gut undoubtedly influences absorption by inducing macroscopic mixing (Lentle et al. 2005; Macagno and Christensen 1980, 1981; Melville et al. 1975) and may also influence the rate of dissolution of particles. Comparisons of in vitro profiles of dissolution of paracetamol with in vivo rates of absorption indicate dissolution does not depend solely on the rate of hydrodynamic flow but also on erosive forces generated during intestinal contraction (Shameem et al. 1995). Contractile activity, being episodic in nature, is unlikely to generate the smooth plug flow conditions which are assumed in some pharmacological models (Oh et al. 1993). Rather, it will induce temporal and spatial variations in flow which will establish a degree of mixing in both the radial and axial directions.

A body of evidence indicates that cellular and neuronal elements in the gut wall are responsive to the physical conditions within the lumen as well as to the levels of certain contained nutrients (Bornstein et al. 2002). Indeed, the significant progress in our understanding of the electrophysiological events that lead to contraction have been derived from in vitro preparations in which contractile activity is induced by maintaining the intraluminal pressure at an elevated level, i.e. the Trendelenberg preparation (Trendelenburg 1917).

The operation of receptors that respond to stretch (Spencer et al. 2002; Spencer et al. 2003), tension from muscle tone (Smith et al. 2003), and touch (Smith and Furness 1988) has been electrophysiologically characterised in various sections of the gut, as has the modulation of receptor discharge with rate of stretch (Won et al. 2005). It is conceivable that integration of the outputs from arrays of such receptors in adjacent segments of the intestine could provide information that is analogous to assessment of rheological characteristics such as apparent viscosity. However, the reduction in the efficiency of absorption of nutrients, such as glucose, following the consumption of viscogenic agents (Dikeman and Fahey 2006) renders the prospect of a reflexly maintained ‘optimality’ (Penry and Jumars 1987) of mixing unlikely. It seems more likely that contractile reflexes serve to optimise onflow and particle dissolution rather than mixing. Thus orchestrated periods of augmented contractile activity during the interval between meals serve to remove residual debris (see the section on the MMC cycle in Chap. 7). Again, receptors in critical locations such as sphincters, e.g. the pyloric sphincter, may provide composite information relating to ‘ease of flow’ (Lentle et al. 2010) and appropriately augment contractile activity in adjacent portions of the gut.

The motor function of the digestive tract may therefore be rationalised as operating to maximise the efficiency of digestion while continuing to function in the face of significant perturbations in the quantity, quality and physical properties of ingested food, i.e. functioning in a robust manner.

1.4 An Evolutionary View

It is commonly assumed that the gastrointestinal ‘fitness’ of an organism is an offshoot of its ability to forage optimally, i.e. that an animal needs to compete with others for nutrients and that part of this competition is to optimise digestibility and nutrient yield (Cochran 1987; Penry and Jumars 1987; Sibly 1981; Sibly and Calow 1986). Viewed in this manner, the optimisation of digestion comprises either a specialisation in the processing of a particular forage or the maintenance of a capability to efficiently digest a range of forages according to their availability. For a particular food, the latter strategy can always be defeated by a specialist strategy but allows the organism sufficient breadth of food choice to allow it to exploit items that are locally abundant (Schoener 1971). The generalist strategy is, in effect, an investment in the likelihood that a particular dietary item will not always be abundant and it trades the specialist ability that enables that item to be digested with the highest efficiency for a ‘generalist’ ability to digest a range of items with a somewhat lower efficiency. Hence it is not reasonable to assume that the digestive efficiency of a dietary generalist, such as humans, is optimal for all dietary items. Models that are predicated on such an assumption, like those based on chemical reactor theory (Penry and Jumars 1987), while affording some insight into general digestive strategies, do not provide a realistic basis for evaluating the processes of digestion of individual dietary items. Similarly, the assumptions are likely to be invalid for animals that exhibit some degree of specialism, e.g. grazers. Thus the ruminant strategy of bovids is more efficient than the hindgut strategy of equids in digesting new grasses, but is less efficient than that of equids in digesting older grasses with high bulk and very low nutrient content on account of limitation in rumen transit time (Hume and Sakaguchi 1991; Illius and Gordon 2009).

An assumption of optimality would also not apply in the case of the digestion of a particular nutrient within a single component of the gut, nor would it be reasonable to assume that digestion within the whole gut is the sum of the digestibilities of a particular nutrient within the various components of the gut in which it undergoes digestion. For example, the digestibility of collagen is likely to be higher in toto following its successive digestion in the stomach and small intestine than the sum of the separate digestibilities of native collagen in these two compartments, owing to the effect of gastric pH and proteases in facilitating the efficiency of pancreatic proteases and overall protein digestibility (Reuterswärd and Fabiansson 1985).

It is likely that a number of components of the gut have functions other than the facilitation of digestion and may in fact decrease digestibility. Thus mucus may function principally to provide lubrication or to act as a barrier to the access of pathogens to the mucus membrane (Cone 2009), either of which actions increases the thickness of the unstirred water layer and the barrier to the diffusion and absorption of nutrients (Levitt et al. 1990).

For all of these reasons, it seems a more practical strategy in evaluating the dynamics that govern the digestion of particular foods, to base models of digestion on real physiological, nutritional and physical data, and to subsequently compare their predictions with those obtained in vivo rather than to work from an assumption of optimality. Such ‘physiological’ models would be useful in evaluating the effect of ingredients that are known to limit digestibility. For example, the adulteration of foods with non-nutrient viscogenic agents is known to reduce the speed of uptake of glucose, i.e. glycaemic index (Dikeman and Fahey 2006). The use of physiological models in evaluating the various effects of these agents on flow and mixing in the small intestine, transit through the stomach, and change in depth of the unstirred water layer could allow the principal mechanism that is responsible for the known antiglycaemic effect to be identified and the use of other ingredients with more specific effects on this parameter to be systematically explored.

References

Amidon GL, Lennernäs H, Shah VP, Crison JR (1995) A theoretical basis for a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res 12:413-420

Bornstein JC, Furness JB, Kunze WAA, Bertrand PP (2002) Enteric reflexes that influence motility. In: Brookes SJH, Costa M (eds) Innervation of the gastrointestinal tract. Taylor & Francis, London

Chen L, Remondetto G, Subirade M (2006) Food protein-based materials as nutraceutical delivery systems. Trends Food Sci Technol 17:272-283

Cochran PA (1987) Optimal digestion in a batch-reactor gut: The analogy to partial prey consumption. Oikos 50:268-270

Cone RA (1999) Mucus. In: Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee JR (eds) Mucosal immunology. Academic Press, San Diego, pp 43-64

Cone RA (2009) Barrier properties of mucus. Adv Drug Deliv Rev 61:75-85

Dikeman CL, Fahey GC (2006) Viscosity as related to dietary fiber: A review. Crit Rev Food Sci Nutr 46:649-663

Fagerholm U, Lennernäs H (1995) Experimental estimation of the effective unstirred water layer thickness in the human jejunum, and its importance in oral drug absorption. Eur J Pharm Sci 3:247-253

Goodacre BC, Murray PJ (1981) A mathematical model of drug absorption. J Clin Pharm Ther 6:117-133

Hume ID, Sakaguchi E (1991) Patterns of digesta flow and digestion in foregut and hindgut fermenters. In: Tsuda T, Saaski Y, Kawashima R (eds) Physiological aspects of digestion and metabolism in ruminants. San Diego, Academic Press, pp 427-451

Illius AW, Gordon I (2009) Prediction of intake and digestion in ruminants by a model of rumen kinetics integrating animal size and plant characteristics. J Agric Sci 116:145-157

Kong F, Singh RP (2009) Digestion of raw and roasted almonds in simulated gastric environment. Food Biophysics 4:365-377

Lehmann U, Robin F (2007) Slowly digestible starch–its structure and health implications: A review. Trends Food Sci Technol 18:346-355

Lentle RG, Hemar Y, Hall CE, Stafford KJ (2005) Periodic fluid extrusion and models of digesta mixing in the intestine of a herbivore, the common brushtail possum (trichosurus vulpecula). J Comp Physiol B175:337-347

Lentle RG, Janssen PWM, Asvarujanon P, Chambers P, Stafford KJ, Hemar Y (2007) High definition mapping of circular and longitudinal motility in the terminal ileum of the brushtail possum trichosurus vulpecula with watery and viscous perfusates. J Comp Physiol B177:543-556

Lentle RG, Janssen PWM, Goh K, Chambers P, Hulls C (2010) Quantification of the effects of the volume and viscosity of gastric contents on antral and fundic activity in the rat stomach maintained ex vivo. Dig Dis Sci DOI 10.1007/s10620-010-1164-y:1-12

Levitt MD, Furne JK, Strocchi A, Anderson BW, Levitt DG (1990) Physiological measurements of luminal stirring in the dog and human small bowel. J Clin Invest 86:1540-1547

Lucas P (2004) Dental functional morphology. Cambridge University Press, Cambridge

Macagno EO, Christensen JM (1980) Fluid mechanics of the duodenum. Annu Rev Fluid Mech 12:139-158

Macagno EO, Christensen JM (1981) Fluid mechanics of gastrointestinal flow. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Raven, New York, pp 335-358

Mandalari G, Faulks R, Rich G, Lo Turco V, Picout D, Lo Curto R, Bisignano G, Dugo P, Dugo G, Waldron K (2008) Release of protein, lipid, and vitamin E from almond seeds during digestion. J Agric Food Chem 56:3409-3416

Melville J, Macagno E, Christensen J (1975) Longitudinal contractions in the duodenum: Their fluid-mechanical function. Am J Physiol 228:1887-1892

Oh DM, Curl RL, Amidon GL (1993) Estimating the fraction dose absorbed from suspensions of poorly soluble compounds in humans: A mathematical model. Pharm Res 10:264-270

Pappenheimer JR (2001) Role of pre-epithelial 'unstirred' layers in absorption of nutrients from the human jejunum. J Membr Biol 179:185-204

Penry DL, Jumars PA (1987) Modeling animal guts as chemical reactors. Am Nat 129:69-96

Reigner BG, Couet W, Guedes J-P, Fourtilla JB, Tozer TN (1990) Saturable rate of cefatrizine absorption after oral administration to humans. J Pharmacokinet Biopharm 18:17-34

Reuterswärd AL, Fabiansson S (1985) In vivo digestibility of insoluble collagen from bovine tendon as influenced by the inhibition of gastric acid secretion. J Food Sci 50:1523-1525

Schoener TW (1971) Theory of feeding strategies. Annu Rev Ecol Syst 2:369-404

Shameem M, Katori N, Aoyagi N, Kojima S (1995) Oral solid controlled release dosage forms: Role of GI-mechanical destructive forces and colonic release in drug absorption under fasted and fed conditions in humans. Pharm Res 12:1049-1054

Sibly RM (1981) Strategies of digestion and defecation. In: Townsend CR, Calow P (eds) Physiological ecology: An evolutionary approach to resource use. Blackwell, Oxford, pp 109-139

Sibly RM, Calow P (1986) Physiological ecology of animals: An evolutionary approach. Blackwell, Oxford

Smith TK, Furness JB (1988) Reflex changes in circular muscle activity elicited by stroking the mucosa: An electrophysiological analysis in the isolated guinea-pig ileum. J Auton Nerv Syst 25:205-218

Smith TK, Oliver GR, Hennig GW, O'Shea DM, Berghe PV, Kang SH, Spencer NJ (2003) A smooth muscle tone-dependent stretch-activated migrating motor pattern in isolated guinea-pig distal colon. J Physiol 551:955-969

Spencer NJ, Hennig GW, Smith TK (2002) A rhythmic motor pattern activated by circumferential stretch in guinea-pig distal colon. J Physiol 545:629-648

Spencer NJ, Hennig GW, Smith TK (2003) Stretch-activated neuronal pathways to longitudinal and circular muscle in guinea pig distal colon. Am J Physiol 284:G231-241

Trendelenburg P (1917) Physiologische und pharmakologische versuche über die dünndarmperistaltik. Naunyn Schmiedebergs Arch Pharmacol 81:55-129

Won KJ, Sanders KM, Ward SM (2005) Interstitial cells of Cajal mediate mechanosensitive responses in the stomach. Proc Natl Acad Sci USA 102:14913-14918

Part I
The Digestion of Particle Suspensions

The food of most animals comprises a mixture of liquids and solids. In microscopic animals this may be directly ingested into the cell. In larger animals such as vertebrates ingested material is processed in the specialised structures and cells of the gastrointestinal tract. The ingested material or digesta generally consists of a mixture of liquid and solid nutritious materials from which nutrients are obtained by solution, digestion and absorption along with unusable detritus which generally persists within the cavity of the digestive system and transits in a solid state. The rate at which digestion and absorption can proceed is limited by a number of physical processes that operate within and between the liquid and solid phases of digesta. The transit of enzymes and soluble nutrients between the surfaces of the digestive organ and the solid or liquid substrate is influenced by the processes of diffusion, advection and mixing. The rate of solution and digestion of solid substrates is limited by their surface area and by the physical nature of the interacting surfaces. The operation of these physical processes is rendered complex as the digesta traverse the physical constraints of the digestive system and as the relative proportion of digestible material declines and that of indigestible residues increase. Hence the physical properties of digesta and the concomitant behaviour of its liquid and solid components may impair or facilitate the bulk mixing of liquid digesta (Anfinsen and Tungland 2005), promote the differential onflow of solid or liquid elements of digesta from a particular segment of the gut (Wolf et al. 2002) and influence the ease with which aggregated masses of particulate solids are deformed and the liquid phase is expelled (Ramkumar and Schulze 2005). Further, the physical characteristics of the solid elements of digesta may impair or facilitate the access of digestive secretions to solid nutrient substrates contained within the particle and the subsequent egress of the soluble products of digestion from the particle (Aguilera 2005).

In the following section we examine those properties of the particulate fraction of digesta that influence the physical processes of their digestion. In this respect we consider ‘particles’ in their broadest sense as any discreet assembly of material that is not in solution in the aqueous phase. Hence we include aggregates of hydrophobic materials such as globules, emulsions and microemulsions as well as suspensions of solid material including micro and nanoparticles.

Firstly, we examine general aspects of the physical structure and digestion of individual digesta particles, including effects that arise from the heterogeneity of particle composition and from the size of particles. Secondly, we consider the digestion of specific components of heterogeneous particles, specifically carbohydrates, protein and fat. Finally, we examine the physical properties of particle suspensions and how these affect the mass transfer of released nutrients within the gut lumen.

Chapter 2
The Microstructure and Digestion of Particles

Contents

2.1 Introduction

A majority of ingested solid materials, both food and pharmaceuticals, are heterogeneous at the microscopic scale. Most natural foods have a cellular structure and incorporate regions of differing composition within the cells, while processed foods such as bread and cheese derive much of their consistency from a three-dimensional network or matrix of solid materials. Most oral dosage forms of pharmaceuticals consist of compacted mixtures of powdered ingredients which include the active components and various excipients. The spatial disposition and physical properties of the components of such microstructures can have a significant influence on the processes involved in the solution and digestion of these particles.

We begin by examining the modes of erosion and the rate-determining processes that govern the digestion of particles with comparatively simple microstructures

R. G. Lentle, P. W. M. Janssen, *The Physical Processes of Digestion*,
DOI 10.1007/978-1-4419-9449-3_2,

such as processed foods, controlled-release drug formulations and microencapsulated flavours. The principal processes we examine are classified as diffusion- or degradation-limited, although other processes have been identified (Pothakamury and Barbosa-Cánovas 1995). We move on to examine the digestion of more complex heterogeneous particles that are more similar to particles derived from natural foods and explore how these may be modified by food processing. Finally, we touch on the modelling of the effect of digestive processes on particulate digesta.

2.2 Physical and Chemical Processes of Particle Digestion

2.2.1 Particle Erosion and Fragmentation

Although the bulk of comminution of solid food occurs during mastication, there is generally a further reduction in particle size as the fragments traverse components of the gastrointestinal tract, notably the stomach. However, the forces applied to food particles that result either from fluid shear or from direct compression by the gut walls are small in comparison to those applied by the teeth. Thus the maximum bite force exerted by the human jaw has been reported to be around 400 N (Lucas 2004), whereas the maximum force exerted by the gastric antrum has been estimated at only 0.65 N (Marciani et al. 2001). The lower magnitude of the latter would indicate that the facture strength of a newly ingested food particle must undergo significant reduction from the action of gastric secretions before the particle can be further fragmented.

The size of ingested particles is also reduced by a process of erosion, which can be classified as either surface or bulk (Fig. 2.1). In surface erosion, the rate of ero-

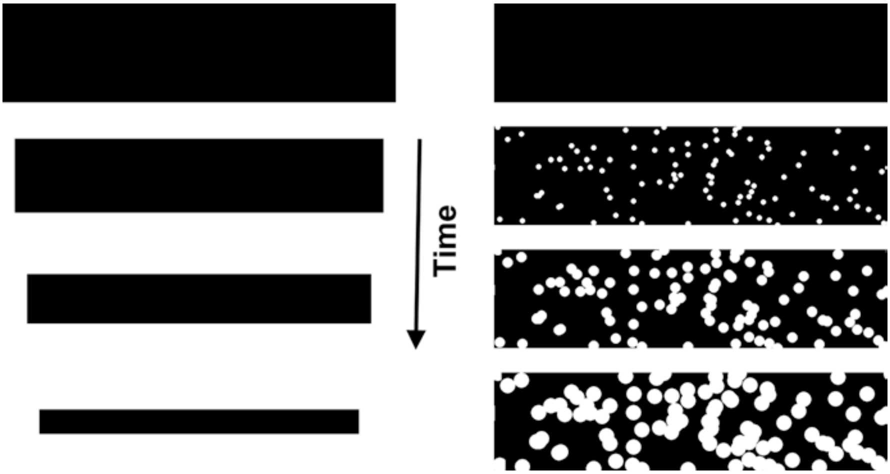


Fig. 2.1 Comparison of surface *(left)* and bulk *(right)* erosion of a particle

sion of the surface of the particle matrix exceeds the rate at which water permeates into it resulting in digestion being a predominantly surface-based process. Thus in the dissolution of a highly soluble particle, material is released into the luminal solution from the outside of the particle and the boundary of the particle moves smoothly inwards as the mass of the particle is progressively reduced. Conversely in bulk erosion, water molecules are able to permeate into the interior of the particle matrix at a rate that exceeds the rate at which it is eroded, and dissolution or digestion occurs within the particle (Langer 1990; Uhrich et al. 1999). Moreover, the boundary of the particle may be increased as inward erosion extends its surface area.

The polymers used in controlled-release drug formulations tend to be classified as surface or bulk eroding. However, some materials appear to change from surface to bulk eroding as the particle size is reduced (Burkersroda et al. 2002).

2.2.2 Diffusion-Limited Processes

Simple immediate-release pharmaceutical preparations may approximate a solid particle that dissolves unhindered across an evenly moving front in accordance with the Noyes–Whitney equation (Brunner 1904; Nernst 1904). The dissolution rate in such a situation is controlled by diffusion across a boundary layer:

$$\frac{dX_d}{dt} = \frac{A\,D}{\delta}(C_s - C_b)$$

where X_d is the amount of the substance in solution, A is the effective surface area of the particle, D is the diffusion coefficient or diffusivity of the substance through the boundary layer fluid, δ is the effective diffusion boundary layer thickness immediately adjacent to the dissolving surface, C_s is the saturation solubility of the substance under lumen conditions and C_b is the concentration of the substance in the bulk luminal solution.

In mass transfer terms, the driving force is the concentration difference between liquid at the particle surface and in the bulk. A highly soluble material will have a high C_s value and hence a high dissolution rate. For a poorly soluble material, C_b will increase and approach C_s due to the limited volume of solvent available resulting in a reduction in the rate of dissolution. As dissolution progresses the surface area diminishes, hence the dissolution rate of a simple particulate material is expected to decrease as it moves through the digestive tract. The pH in the boundary layer may also influence the solubility of a substance by influencing its surface charge (Dressman et al. 1998).

For a viscous fluid moving across any surface, the fluid velocity at the solid–liquid interface is usually zero (i.e. the no-slip condition) and there is a layer of fluid close to the wall in which the velocity is reduced from that of the surrounding bulk fluid. The Nernst diffusion layer *(δ)* is a hypothetical stationary layer that has an equivalent mass transfer resistance and for technical applications typically has a thickness in the range 5–100 µm. The thickness of the layer, and hence the diffusion rate, depends on the velocity of the bulk luminal solution relative to the particle and on the viscosity of that solution (Khoury et al. 1988). Hence when attempting to simulate mass transfer conditions in the lumen of the gut using the United States Pharmacopeia (USP) paddle dissolution apparatus, it is necessary to carefully select the rotational speed to simulate the appropriate hydrodynamic conditions in the requisite component of the gastrointestinal tract (Scholz et al. 2003).

While the Noyes–Whitney equation was developed for the dissolution of homogenous particles, the general form may be applicable to more complex particles and has been widely used in the pharmacological field. Thus for a particle consisting of a finely dispersed phase in a matrix of soluble material, the release rate of the dispersed phase is directly related to the dissolution rate of the matrix. Similarly, for soluble particles that are each surrounded by a layer of insoluble material, i.e. as is the case in some reservoir-type controlled-release pharmaceutical formulations, the same equation can be applied to the dissolution of the inner particle by substituting the thickness of the outer layer for δ and using the diffusivity of the inner material through the outer layer.

Diffusion is a key process, not only in the digestion of solid particles, but also in the mass transfer of released nutrient to the epithelium. The diffusivity of molecules or very small particles through a liquid is often estimated using the Stokes–Einstein equation (Cussler 1997), which indicates that the diffusivity should be inversely related to the diameter of the particle and also to the viscosity of the liquid surrounding the particle. Therefore, one would expect the diffusivity of a monomer to be higher than that of an oligomer, and the diffusivity of a molecule to be higher than that of a micelle. It is also noteworthy that the diffusivity of a particle is affected by the microrheology of the surrounding liquid rather than the bulk rheology of the luminal fluid.

Digesta commonly comprise suspensions of heterogeneous particles that often contain or absorb materials that form hydrogels. We are particularly interested in the diffusion of nutrient molecules through such suspensions or through a layer of hydrogel. The suspension case can be considered as analogous to one of diffusion through a porous media in which the diffusivity is reduced from that in the pure liquid, both by the reduced cross-sectional area of the flow channels, and the extra length and winding of the flow path, i.e. tortuosity. One way of dealing with this analytically is to calculate the 'effective diffusivity' by adjusting the diffusivity in the liquid for porosity and tortuosity (Geankoplis 2003). In cases where hydrogels are formed, diffusion may also be hindered by other factors, including the size of the nutrient molecules relative to the distance between adjacent polymer chains, the mobility of the polymer chains (influenced by the density of their cross-linking), and the interaction of charged nutrient molecules with charged groups on the polymer chains (Amsden 1998).

Particles containing appropriate polymers may become surrounded by hydrogels as constituent polymers become hydrated, or particles may become coated with an adherent layer of secreted mucins. The same equation as was developed for the dissolution of simple particles can be adapted for such situations by substituting the thickness of the outer hydrogel layer for the Nernst layer δ. The diffusivity would be dependent on the nature of the hydrogel, and hence the rate of release of a pharmaceutical ingredient can be controlled by manipulating the degree of cross-linking in the polymer.

On occasion, the nutrient or active ingredient is held within a polymeric matrix through which it is unable to diffuse due to the latter being in the glassy state. In such a ‘swelling-limited’ process, the rate of solution or digestion may be limited by the rate of penetration of water into the matrix, causing it to swell and transform from a glassy to a rubbery state. A nutrient or active pharmacological ingredient in such a particle can diffuse much more quickly through the polymer to the surrounding liquid once the polymer has swollen (Fan and Singh 1989). Hydrophilic polymers, such as hydroxypropyl methylcellulose (HPMC), are typically used in controlled-release drug delivery devices in which the release is controlled by the kinetics of the polymer chain disentanglement (Arifin et al. 2006).

2.2.3 Degradation-Limited Processes

In addition to the physical processes dealt with above, the breakdown of many food particles includes chemical degradation, predominantly by enzymatic reactions, but also by acidic hydrolysis. Enzymatic reactions are often assumed to follow Michaelis–Menten (1913) kinetics. According to this model, at low concentrations of substrate, the rate of the reaction is proportional to the concentration of the substrate, i.e. first order, but the rate becomes independent of the substrate concentration at high concentrations of substrate, i.e. zero order. In the latter case, the reaction rate depends solely upon the rate of dissociation of the enzyme from the product. In most situations, the concentration of enzyme is much lower than that of the substrate, and hence the reaction rate is first order with respect to enzyme concentration.

It is important to consider whether the enzymatic reactions that take place during digestion are homogenous or heterogeneous, i.e. whether the reaction is taking place in a single phase or involves components distributed between two or more phases (Levenspiel 1999). If the enzymatic reaction involves dissolved nutrients, the reaction is said to be homogeneous and its rate will be determined by the bulk nutrient and enzyme concentrations. Where components of solid particles are being digested by dissolved enzymes, i.e. a heterogeneous reaction, its rate is influenced by the concentration of enzyme at the surface of the particle and by the particle’s active surface area. Hence, provided that sufficient enzyme is present, digestion of fat emulsions in the stomach and duodenum would be expected to proceed at a greater rate than that of simple mixtures of fat and water due to the larger area of the oil-water interface in the emulsion (Marciani et al. 2008).

Two types of system have been synthesised that result in degradation-limited release of drugs; those in which the active ingredient is bound to a hydrogel network by cleavable links and those in which the active ingredient is physically trapped in a network that must be degraded by surface erosion for the ingredient to be liberated (Lin and Metters 2006). If the reactions involved in such release are non-enzymatic, the degradation of the matrix can be assumed to follow simple first-order kinetics. Analogous situations may exist where components of particles of natural foods are similarly digested.

It has been suggested that the effects of differing degrees of geometric constraint on the diffusion of soluble products from, and the diffusion of enzymes into, the matrices of the broadly heterogeneous range of particles that are found in digesta will cause the kinetics of enzymatic degradation to be similarly heterogeneous and vary in a manner that can best be described in terms of fractal concepts (Macheras and Argyrakis 1997). More work is required to characterise the extent and degree of influence of such heterogeneity on the digestion of different foods, and to confirm whether the digestion reactions follow fractal kinetics.

2.3 Digestion of Heterogeneous Particles Derived from Food

While considerable work has been carried out on the dynamics of erosion and degradation in drug delivery systems (Langer 1990), relatively little has been conducted on the dynamics of digestion of the matrices of food particles. Food particles, being derived from heterogeneous foods by a randomly oriented process of fracture during mastication, are similarly heterogeneous. The masses of these particles may be further reduced during their transit through the gut by fragmentation and by heterogeneously distributed erosion and degradation.

The digestion of heterogeneous food particles can lead to the accumulation of insoluble components at their surfaces which impede the solution and diffusion of any contained nutrients. Similarly, soluble viscogenic material can dissolve and disperse through the aqueous phase, inhibiting both the solution and the diffusion of a soluble nutrient. Further, the presence and persistence of intracellular or extracellular structures that envelop or otherwise sequester particulate nutrients (which we shall term lamellar barriers) may impair their solution, digestion and absorption.

2.3.1 Heterogeneity in Foods

Natural foods are generally heterogeneous and can be broadly classified into four categories (Parada and Aguilera 2007): fleshy plant material; encapsulated plant embryos, e.g. grains and pulses; animal tissues and milk. The first three of these comprise heterogeneous arrays of cells with heterogeneously distributed contents. Although the fourth appears macroscopically to be homogenous, it comprises a fat emulsion dispersed in a continuous aqueous phase, which also contains dispersed colloids. Such a level of heterogeneity is sufficient to complicate the digestion of the contained fat, which must be digested at the oil-water interface. The colloids dispersed in the aqueous phase may interact with this interface, the degree of interaction varying with the state of digestion of the colloidal material (see Chap. 4).

Plant-based foods contain significant amounts of extracellular material, such as cellulose, hemicellulose, pectin and lignin components, that are organised to perform particular mechanical functions (Ellis et al. 2004; Vincent 1990). This extracellular material generally includes a matrix which surrounds the cells and acts as a barrier to the permeation of digestive enzymes (Guillon and Champ 2000). The microstructure of these extracellular materials may influence the ease of such permeation. Thus evidence suggests that the extent to which dried fibres are permeated by the aqueous phase of digesta (Guillon and Champ 2000) is influenced by the size and volume of pores therein (Guillon et al. 1998).

The characteristics of the structural matrices within particular foods are known to influence the bioavailability of contained nutrients including minerals (Brouns and Vermeer 2000; Moretti et al. 2006), carotenes (Zhou et al. 1996) and lycopene (Adetayo and Omoni 2005). Consequently, it is increasingly recognised that the rate, site and extent of absorption of nutrients may be controlled by altering the rate of degradation, dissolution and permeability of the food matrix that contains them (Chen et al. 2006; McClements et al. 2008; Parada and Aguilera 2007). Pharmaceutical and food engineering studies have identified a number of parameters that influence the ease with which such matrices may be eroded and degraded, notably their hydrophobicity (Chen et al. 2006; Langer 1990), the tortuosity of potential routes for aqueous permeation (Aguilera 2005), and the transit of water through lamellar barriers by osmosis (Santus and Baker 1995).

2.3.2 Digestion of Cellular Plant Material

There are two potential semi-permeable barriers to the digestion of the contents of plant cells: the hydrophilic extracellular wall, which comprises a mixture of cellulose, hemicellulose and pectin; and the cell or plasma membrane. The pore sizes of normal non-lignified extracellular walls lie in the range 3.5–5.5 nm, which would allow the permeation of small molecules such as simple sugars, amino acids and proteins up to approximately 10–50 kDa in size (Brett and Waldron 1996). Permeation of the extracellular wall by digestive secretions may cause the walls to swell and the size of these pores to increase. Thus transmission electron microscopy of almond fragments showed that significant swelling (i.e. sixfold) of intact cell walls occurred after 12 h digestion in vivo which was postulated to allow access of bile and lipolytic enzymes (Mandalari et al. 2008). Subsequent transit of the permeating material through the lipid bilayer of the cell membrane would depend on its lipophilicity and the integrity of cell membrane.

The cellular structure within particles of digesta that are derived from a number of plant-based foods is known to persist after their transit through the human gut (Edwards et al. 2002; Faulks and Southon 2005; Mandalari et al. 2008; Parada and Aguilera 2007; van het Hof et al. 2000a, b, 2007). The fact that the release of carotenoids from such foods is significantly augmented when their cellular components

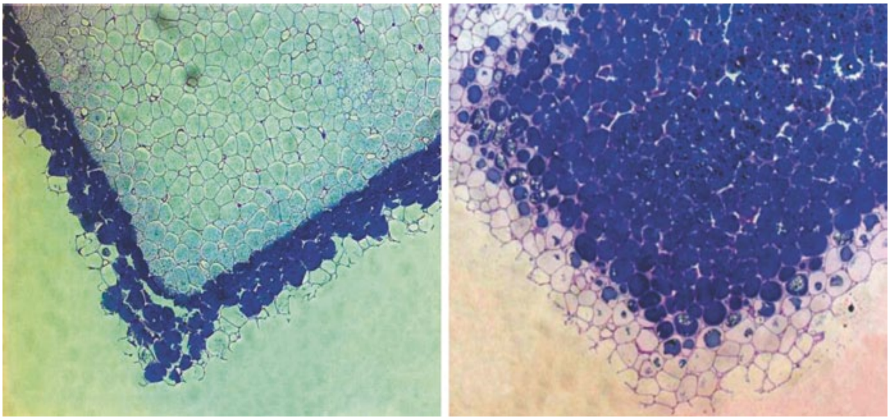


Fig. 2.2 Light microscopy section through a 2-mm cube of natural almond after 3 h *(left)* and 12 h *(right)* of in vivo digestion. A toluidine blue stain has been used to visualise the penetration of luminal fluid into the interior of the particle. A layer of empty cell wall material surrounds the particle increasing in thickness with digestion time. (Mandalari et al. 2008)

have been disrupted (Parada and Aguilera 2007) indicates that elements in the walls of these cells limit their permeability to digestive fluids during transit through the gut. The digestion of almonds, both natural and roasted, is one of the better-studied examples of such behaviour (Ellis et al. 2004; Kong and Singh 2009a; Mandalari et al. 2008). Lipids, the main macronutrient component of almonds, are stored therein as small intracellular oil bodies. Such is the integrity of the cellular structure of almonds that chewing or mechanical processing generally damages only the cell walls situated around the perimeter of the particles allowing any contained oil to permeate to the particle surfaces (Ellis et al. 2004). While this free oil can readily undergo the normal digestive processes of emulsification and subsequent lipolysis (see Chap. 5), these processes appear to be hindered where oil is contained in areas with undamaged cell structure. Comparative microscopy of particles of natural almonds that had undergone digestion in vitro or in vivo for differing lengths of time showed that the time taken for them to be penetrated by digestive fluids was close to the total residence times of the particles within the foregut (Mandalari et al. 2008) (Fig. 2.2). The same work found that the digestion of the contents of intact cells situated below the surfaces of these particles was governed by the rate of permeation and enzymatic degradation of their cellulosic walls, the process being exemplified by the progressive swelling of these walls.

2.3.3 *Lamellar Barriers in Plant Tissues*

Some plants have evolved specific morphological components that function as lamellar barriers and enable them to resist digestion. Such barriers can be found at a number of morphological levels. They can take the form of a coating on the surfaces of starch granules, or a coating that replaces cellular structures in seeds germs, and may also be formed to protect important parts of the plant (see Chap. 3). These protective layers as well as those associated with cell walls may remain intact within heterogeneous particles of digesta, sequestering nutrients and rendering them more resistant to digestion. For instance, the seed coat of almonds is largely undamaged by intestinal secretions or by bacterial fermentation (Ellis et al. 2004).

Similar lamellar barriers may be deliberately or inadvertently produced in processed foods. Coatings of biopolymers, such as egg white, soybean and whey proteins, are known to impede solution or digestion of sequestered nutrients (Chen et al. 2006) by virtue of the polymer's hydrophobicity (Chen et al. 2006; Langer 1990) or tortuosity (Aguilera 2005). For example, hydrogels formed from processed food proteins have been shown to protect the antioxidant α-tocopherol from degradation in the digestive tract (Chen et al. 2006). Similarly, coatings of naturally occurring non-starch polysaccharides have been shown to protect solid dosage forms of drug from solution and digestion in the proximal gut, and allow them to enter the colon (Sinha and Kumria 2001).

2.3.4 Erosion Modes of Food Particles

Work examining the kinetics of breakdown of representative foods in an ex vivo system that reproduces the salivary and intragastric phases of digestion under conditions of controlled shear, indicates that both surface and bulk erosion can occur, but that the relative contributions of the two types of erosion vary according to the food and its manner of processing (Kong and Singh 2008, 2009b). Thus the masses of pieces of uncooked carrot were reported to decline sigmoidally over time while that of pieces of cooked carrot and of ham declined exponentially. The initial slow decline in the mass of raw carrot was correlated with the high strength of the matrix of cell walls within. Progressively reducing the strength of this matrix by boiling the pieces of carrot for increasingly longer periods of time caused them to disintegrate more rapidly and the decline in their mass to become more exponential in form. This indicated that the strength of the raw carrot matrix was such that disintegration could only occur after an initial period of softening by permeating digestive juices. When the matrix was made softer, it disintegrated by surface erosion which did not depend upon the process of permeation.

When softer materials such as ham were exposed to the same enzymatic cycle in the absence of any applied force, permeation progressed deeply into the matrix but no bulk erosion took place. It appears that, within the stomach at least, the reduction in the size of some types of food particles requires the physical disruption of any insoluble matrix that remains after permeation, solution and degradation of some of its structural constituents by the action of enzymes. In some cases, the bulk of the matrix is composed of the principal nutrient and is itself soluble, e.g. the matrices of particles of beef jerky disintegrate once they are dissolved but this disintegration is accelerated by the addition of pepsin and an appropriate pH (Kong and Singh 2009b).

It is important to note that the digestion of particles generated from natural foods may depart significantly from the particle erosion dynamics of controlled-release drug formulations (Langer 1990) due to their more heterogeneous structure. Thus surface erosion appears to dominate the disintegration of particles generated from natural almonds, even though the rate of this erosion is slower than the rate at which digestive juices permeate into their matrix (Mandalari et al. 2008). In this case, it seems that only cells on the periphery of the particle are subject to mechanical forces of a sufficient strength to abrupt them.

Given that the solubility of an element in the particulate matrix may vary according to local conditions, e.g. pH, those physical properties of a particle which cause it to remain within a particular segment of the gut for longer periods may influence the type and duration of matrix degradation. For example, as in the case of floating drug delivery systems (Singh and Kim 2000), buoyant particles may undergo longer periods of acidic matrix erosion and degradation before leaving the stomach than do denser particles.

2.3.5 Effects of Cooking and Food Processing on Particle Digestion

The cooking of many foods that are common to the Western diet improves their digestibility and reduces the need for mastication (Farrell 1956). The cooking of vegetables and fruits brings about an initial rapid reduction and subsequent slower decline in their firmness (Huang and Bourne 1983). The rapid initial phase appears to be due to the disruption of cell membranes and consequent loss of turgor (Greve et al. 1994). The slower subsequent phase is thought to result from degradation and solution of pectic polysaccharides causing weakening of the middle lamella and a reduction in intercellular adhesion (Van Buggenhout et al. 2009). As a consequence, the predominant mode of mechanical failure appears to change from one of cell rupture to one of cell separation.

Pieces of raw carrot are reported to retain a recognisable cellular form after they have traversed the human gastrointestinal tract (Farrell 1956). This may be partly due to the microstructure of carrot, the small (50 μm) tightly packed cells and lack of intercellular air spaces reducing the ease with which luminal liquids can permeate the matrix. The modification of this structure by cooking, augments disintegration digestion in vitro (Kong and Singh 2008) and in vivo. Hence the threefold increase in plasma concentration of β-carotenes following consumption of an aliquot of cooked rather than raw carrot probably results from disruption of cell membranes and weakening of the matrix structure (Rock et al. 1998).

Cellular structure and integrity was found to be preserved in faecal particles obtained following the consumption of raw almonds that had been processed in a laboratory blender (Ellis et al. 2004). However, the roasting of almonds can promote the access of enzymes to and the efflux of products from the component cells of almond particles via channels and voids created by the shrinking of component cells and the venting of steam (Kong and Singh 2009a).

The cooking and grinding of meat similarly increases the rate of digestion and assimilation by facilitating the access of gastric acids and proteolytic enzymes (Boback et al. 2007). Cooking promotes the destruction of cell membranes, the aggregation and gelling of sarcoplasmic proteins, and the denaturation of fibrous muscle proteins including collagen (Tornberg 2005). Denaturation of myofibrillar proteins commences at around 40 °C, and initially leads to the contraction and toughening of the meat. However, the denaturation of collagen, the main component of the surrounding connective tissue, starts at around 55–60 °C its components being converted into gelatine unless they are stabilised by heat-resistant intermolecular bonds. This latter process causes the meat to become increasingly tender and more easily fragmented.

2.4 Effects of Size on the Digestion of Particles

Given that the dissolution and digestion of a solid particle, either by surface or by bulk erosion, is mediated via a surface, the rate at which these processes proceed should be related to its surface area. A finely ground solid material has a high specific surface area, and it is expected that the contained nutrients will be released into the luminal liquid more rapidly than from a coarser material. However, the rate of digestion or dissolution may not be proportional to surface area when debris accumulates on the particle surface or when fine particles adhere to each other reducing the exposed area.

Although a body of work documents the positive effect of increasing surface area on digestibility, little work has explored the boundaries of this relationship. It has been found that the reduction of food particle size by mastication (Read et al. 2007) or physical processing (Heaton et al. 1988) generally leads to a higher peak in plasma glucose levels. However, care is required in the interpretation of such results as the effect of particle size tends to be confounded with the effect of disrupting the structure of heterogeneous particles. Further, nutrient digestibilities of corn feed by pigs have been found to increase as particle size is progressively reduced from 1,000 µm to 400 µm (Wondra et al. 1995). This would seem to indicate that the rate at which particles are digested increases as particle size is decreased, but the mathematical relationship between these two parameters is not described and may not be linear. Hence in situations where finer particles have a greater tendency to adhere to each other, the relationship may be curvilinear particularly in cases where particulate aggregates have untoward effects. In this respect, it is noteworthy that finely ground feeds are reported to promote gastric ulceration in the pars oesophagea of pigs (Nielsen and Ingvartsen 2000) and that the feeding of finely ground grains to broiler chicks causes foregut impaction and increased death rate (A. Amerah pers. comm.).

There are limits in the capacity of animals to reduce the particle sizes of natural foods, notably in the process of human mastication (Lucas 2004) and in the forces applied during gastric trituration (see Chap. 9). Similarly, there is an economic limit to the fineness of grinding in preparing stock feeds due to the mechanical energy requirement (Wondra et al. 1995). Except for specifically engineered particles, these limits are likely to preclude the generation of particles that are capable of transiting the mucus layer (see Chap. 10).

2.4.1 Solubility and Absorption of Nanoparticles

Smaller particles have a greater apparent solubility than larger particles due to the increase in surface energy when the surface of a particle is more curved, i.e. is smaller, a phenomenon termed the Ostwald–Freundlich effect (Acosta 2009; Müller et al. 1999). This effect is more pronounced at very small particle sizes and could be expected to significantly augment the apparent solubility of nanoparticles in digesta. In fact, there is a significant enhancement of the apparent solubility and uptake (i.e. bioavailability) of poorly soluble materials at particle sizes around 500 nm, a size greater than that at which the Ostwald–Freundlich phenomenon produces a significant effect (Acosta 2009). This indicates that the uptake of material from nano-sized digesta particles may be augmented by other means, such as entrapment and dissolution in the mucus layer of the gut wall, or by their direct cellular uptake (Acosta 2009; Horn and Rieger 2001).

Direct uptake may occur by three possible routes: via enterocytes; via specialised M cells in the lining mucosa; or by passage across the junctions between adjacent enterocytes, i.e. paracellular transport (Desai et al. 1996; Hussain et al. 2001). It is thought that the latter route would be least effective in this regard given that the space between adjacent cells ranges from 0.3 to 1 nm (Acosta 2009). However, it is noteworthy that a variety of much larger particles (3–100 µm) have been observed to transit the lymphatic lacteals within villi, including intact starch granules (Herbst 1844; Volkheimer et al. 1968), and particles of coal (Hussain et al. 2001). While it is known that the integrity of tight junctions may vary with the inflammatory status of the adjacent enterocytes (Nusrat et al. 2000), it is difficult to envisage a mechanism that would allow the paracellular or transcellular passage of material of such size and composition, and no such mechanism has been described.

The uptake of inert nano-sized particles in the small intestine has been shown to occur via all three routes (Aprahamian et al. 1987). Thus direct absorption of particles is reported in the colon (Jani et al. 1992a, b) and rectum (Fukui et al. 1987). However, the proportions of nanoparticles that are absorbed via such routes vary according to the material of which they are composed and their surface characteristics, e.g. nanoparticles of radioactive iridium do not appear to be absorbed in the gut (Kreyling et al. 2002).

The uptake of nanoparticles is also influenced by the ease with which they transit the mucus layer and the cytosol within the enterocyte. For instance, the presence of a net positive or negative surface charge (Jani et al. 1989; Norris and Sinko 1997a) impairs the transit of nanoparticles through the mucus layer while surface hydrophobicity is reported to facilitate it (Norris and Sinko 1997a, b). Conversely, surface hydrophobicity impairs the passage of nanoparticles through the enterocyte cytosol (Norris and Sinko 1997b) (see the mucus section in Chap. 10).

A body of evidence indicates that the physicochemical properties of materials may change when they are reduced to nano-sized particles (Nel et al. 2006). This may be of no consequence when nanoparticles that are formed from normal dietary components are absorbed (Acosta 2009), but there is some evidence suggesting that nanoparticles formed from substances not normally encountered in the diet may persist after absorption. Thus polystyrene nanoparticles enter the lymphatic system following absorption and are subsequently redistributed to other systems (Hillery et al. 1994; Jani et al. 1990). The accumulation of such nanoparticles is reported to be correlated with pro-inflammatory change, the perturbation of mitochondrial function and the generation of reactive oxygen species (Nel et al. 2006).

Experiments with coated nanoparticles indicate that barriers to the ingress of digestive secretions and the egress of digestive products may act at a molecular level. Thus the release of soluble material from nanoparticles is retarded by coating them with native β-lactoglobulin (Chen et al. 2006). The latter material is thought to resist the action of pepsin as the hydrophobic amino acids, which are attacked by this enzyme, are internalised within the lactoglobulin molecule, and hence are inaccessible (Chen et al. 2006; Chen and Subirade 2005).

2.5 Modelling of Combined Digestion and Absorption Processes

The pharmaceutical industry has developed increasingly complex models of the processes of digestion and absorption (Yu et al. 1996), which are designed to assess the behaviour of novel formulations of therapeutic substances (Parrott and Lave 2008). Early models, such as those based on mass balance (Oh et al. 1993), assumed a steady-state, and hence were only capable of predicting overall absorption. These were superseded by dynamic models, which could predict the rate of absorption and were developed in two basic forms: dispersion models, which treated the gut as a single tube with varying properties along its length; and compartmental models, which partitioned the lumen into approximately 7–9 sequential compartments (Huang et al. 2009).

In principle, the same modelling techniques could be applied to the digestion and absorption of food (Juillet et al. 2006). Such adaptation would require data on the rate of transfer of nutrient mass from the particle to the surrounding fluid phase, as well as the rate of absorption of the nutrient across the epithelium. The mass transfer of nutrients from heterogeneous particles derived from food may be more complex than in typical pharmaceutical preparations owing to the greater number of potentially rate-determining processes that are involved. In particular, the current pharmaceutical models do not appear to deal explicitly with mass transfer across the lumen. This is more likely to be a limiting factor when dealing with suspensions that contain high volumes of food particles, and are pseudoplastic and viscous, rather than pharmaceuticals, which are often treated as single particles in watery digesta.

2.5.1 Mass Balance Approach

These simple models perform a mass balance over the small intestine and combine this with algebraic expressions for dissolution and absorption (Amidon et al. 1995; Oh et al. 1993). The rate of onflow from the stomach and the composition of its contents are assumed to be constant, while the small intestine is treated as a cylindrical tube. The resultant models indicate that the fraction of substances absorbed from homogenous suspensions of particles is governed largely by three dimensionless numbers: the absorption number (An), the ratio of the absorption rate to the rate of perfusion, i.e. the axial convection rate; the dose number (Do), the ratio of the concentration of the substance in the digesta to the solubility of the substance in digesta; and the dissolution number (Dn), the ratio of the residence time of the digesta in the segment to the time for the particles to dissolve (Oh et al. 1993).

These models can be useful in understanding the factors determining the extent of drug absorption. For example, the models have been used to predict and compare the extent of absorption of griseofulvin and digoxin. Both drugs are easily absorbed and have similar solubilities, yet a greater fraction of digoxin is expected to be absorbed. This is due to the large therapeutic dosage of griseofulvin relative to its solubility giving a comparatively high value for Do. Grinding digoxin to a very fine particle size, i.e. increasing Dn, can be expected to result in almost complete absorption, whereas similar grinding of griseofulvin will produce only a marginal increase.

2.5.2 Compartmental and Absorption Transit Models

The first compartmental absorption and transit (CAT) model divided the small intestine into seven sequential compartments, i.e. segments, each with equal residence time (Yu and Amidon 1999). The model assumed that absorption from the stomach and colon was negligible, that transport across the intestinal wall was passive, and that dissolution was instantaneous. The interfacing of the CAT model to a pharmacokinetic model allowed the plasma concentration profile of a therapeutic substance to be predicted following oral dosage. The models have been progressively refined to create the advanced compartmental absorption and transit (ACAT) model (Fig. 2.3) which incorporates controlled-release dosage forms, non-linear absorption kinetics (e.g. Michaelis–Menten), polydisperse particle sizes, gastric emptying, etc. (Agoram et al. 2001; Huang et al. 2009).

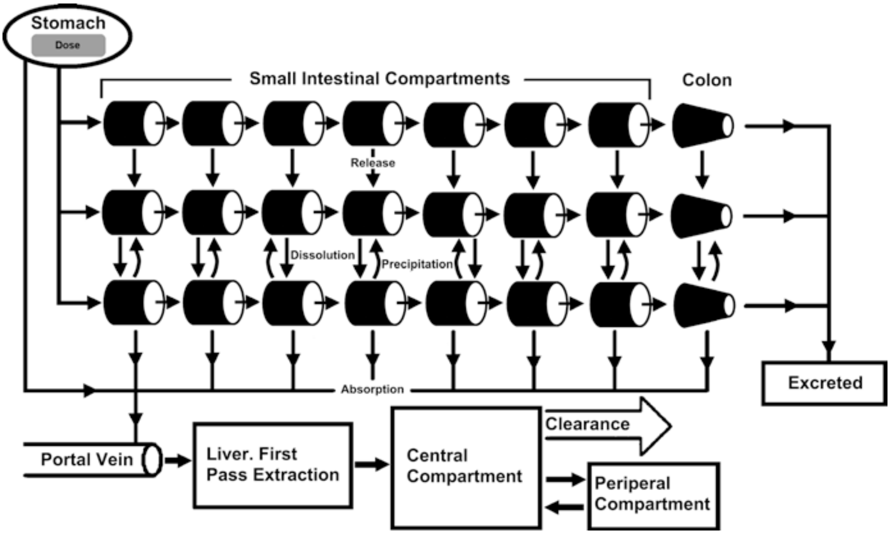


Fig. 2.3 ACAT model with separate compartments for the controlled-release dosage form, the released solid, and the luminal solution for each of the nine segments along the gastrointestinal tract. (Agoram et al. 2001)

A typical application of CAT models considers both dissolution and absorption, and can incorporate data obtained from in vitro and in vivo experimentation. For instance, dissolution data may be obtained from a USP apparatus, while permeability may be determined across a monolayer of Caco2 cells. Recent work has been directed toward incorporating the effect of food intake into the model by obtaining dissolution data under biorelevant conditions (Parrott et al. 2009).

References

Acosta E (2009) Bioavailability of nanoparticles in nutrient and nutraceutical delivery. Curr Opin Colloid Interface Sci 14:3-15

Adetayo O, Omoni R (2005) The anti-carcinogenic and anti-atherogenic effects of lycopene: A review. Trends Food Sci Technol 16:344-350

Agoram B, Woltosz WS, Bolger MB (2001) Predicting the impact of physiological and biochemical processes on oral drug bioavailability. Adv Drug Deliv Rev 50:S41-S67

Aguilera J (2005) Why food microstructure? J Food Eng 67:3-11

Amidon GL, Lennernäs H, Shah VP, Crison JR (1995) A theoretical basis for a biopharmaceutic drug classification: The correlation of *in vitro* drug product dissolution and in vivo bioavailability. Pharm Res 12:413-420

Amsden B (1998) Solute diffusion within hydrogels. Mechanisms and models. Macromolecules 31:8382-8395

Aprahamian M, Michel C, Humbert W, Devissaguet JP, Damge C (1987) Transmucosal passage of polyalkylcyanoacrylate nanocapsules as a new drug carrier in the small intestine. Biol Cell 61:69-76

Arifin DY, Lee LY, Wang CH (2006) Mathematical modeling and simulation of drug release from microspheres: Implications to drug delivery systems. Adv Drug Deliv Rev 58:1274-1325

Boback SM, Cox CL, Ott BD, Carmody R, Wrangham RW, Secor SM (2007) Cooking and grinding reduces the cost of meat digestion. Comp Biochem Physiol A 148:651-656

Brett C, Waldron K (1996) Physiology and biochemistry of plant cell walls. Chapman & Hall, London

Brouns F, Vermeer C (2000) Functional food ingredients for reducing the risks of osteoporosis. Trends Food Sci Technol 11:22-33

Brunner E (1904) Reaktionsgeschwindigkeit in heterogenen systemen. Z Phys Chem 47:56-102

Burkersroda F, Schedl L, Göpferich A (2002) Why degradable polymers undergo surface erosion or bulk erosion. Biomaterials 23:4221-4231

Chen L, Remondetto GE, Subirade M (2006) Food protein-based materials as nutraceutical delivery systems. Trends Food Sci Technol 17:272-283

Chen L, Subirade M (2005) Chitosan/β-lactoglobulin core-shell nanoparticles as nutraceutical carriers. Biomaterials 26:6041-6053

Cussler EL (1997) Diffusion: Mass transfer in fluid systems. Cambridge University, Cambridge

Desai MP, Labhasetwar V, Amidon GL, Levy RJ (1996) Gastrointestinal uptake of biodegradable microparticles: Effect of particle size. Pharm Res 13:1838-1845

Dressman JB, Amidon GL, Reppas C, Shah VP (1998) Dissolution testing as a prognostic tool for oral drug absorption: Immediate release dosage forms. Pharm Res 15:11-22

Edwards AJ, Nguyen CH, You CS, Swanson JE, Emenhiser C, Parker RS (2002) α- and β-carotene from a commercial carrot puree are more bioavailable to humans than from boiled-mashed carrots, as determined using an extrinsic stable isotope reference method. J Nutr 132:159-167

Ellis PR, Kendall CWC, Ren Y, Parker C, Pacy JF, Waldron KW, Jenkins DJA (2004) Role of cell walls in the bioaccessibility of lipids in almond seeds. Am J Clin Nutr 80:604-613

Fan L, Singh SK (1989) Controlled release: A quantitative treatment. Springer-Verlag, Berlin

Farrell JH (1956) The effect of mastication on the digestion of food. Br Dent J 100:149-155

Faulks RM, Southon S (2005) Challenges to understanding and measuring carotenoid bioavailability. Biochim Biophys Acta 1740:95-100

Fukui H, Murakami M, Yoshikawa H, Takada K, Muranishi S (1987) Studies on the promoting effect of lipid-surfactant mixed micelles (mm) on intestinal absorption of colloidal particles. Dependence on particle size and administration site. J Pharmacobiodyn 10:236-242

Geankoplis CJ (2003) Transport processes and separation process principles:(includes unit operations). Prentice Hall, New Jersey

Greve LC, Shackel KA, Ahmadi H, McArdle RN, Gohlke JR, Labavitch JM (1994) Impact of heating on carrot firmness: Contribution of cellular turgor. J Agric Food Chem 42:2896-2899

Guillon F, Auffret A, Robertson JA, Thibault JF, Barry JL (1998) Relationships between physical characteristics of sugar-beet fibre and its fermentability by human faecal flora. Carbohydr Polym 37:185-197

Guillon F, Champ M (2000) Structural and physical properties of dietary fibres, and consequences of processing on human physiology. Food Res Int 33:233-245

Heaton KW, Marcus SN, Emmett PM, Bolton CH (1988) Particle size of wheat, maize, and oat test meals: Effects on plasma glucose and insulin responses and on the rate of starch digestion *in vitro*. Am J Clin Nutr 47:675-682

Herbst EFG (1844) Das lymphgefassystem und seine verrichtungen. Vandenhoek u Ruprecht Verlag, Gottingen

Hillery A, Jani P, Florence A (1994) Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60 nm polystyrene particles. J Drug Target 2:151-156

Horn D, Rieger J (2001) Organic nanoparticles in the aqueous phase-theory, experiment, and use. Angew Chem Int Ed Engl 40:4331-4361

Huang W, Lee SL, Yu LX (2009) Mechanistic approaches to predicting oral drug absorption. AAPS J 11:217-224

Huang YT, Bourne MC (1983) Kinetics of thermal softening of vegetables. J Text Stud 14:1-9

Hussain N, Jaitley V, Florence AT (2001) Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. Adv Drug Deliv Rev 50:107-142

Jani P, Halbert GW, Langridge J, Florence AT (1989) The uptake and translocation of latex nanospheres and microspheres after oral administration to rats. J Pharm Pharmacol 41:809-812

Jani P, Halbert GW, Langridge J, Florence AT (1990) Nanoparticle uptake by the rat gastrointestinal mucosa: Quantitation and particle size dependency. J Pharm Pharmacol 42:821-826

Jani PU, Florence AT, McCarthy DE (1992a) Further histological evidence of the gastrointestinal absorption of polystyrene nanospheres in the rat. Int J Pharm 84:245-252

Jani PU, McCarthy DE, Florence AT (1992b) Nanosphere and microsphere uptake via Peyer's patches: Observation of the rate of uptake in the rat after a single oral dose. Int J Pharm 86:239-246

Juillet B, Saccomani MP, Bos C, Gaudichon C, Tomé D, Fouillet H (2006) Conceptual, methodological and computational issues concerning the compartmental modeling of a complex biological system: Postprandial inter-organ metabolism of dietary nitrogen in humans. Math Biosci 204:282-309

Khoury N, Mauger JW, Howard S (1988) Dissolution rate studies from a stationary disk/rotating fluid system. Pharm Res 5:495-500

Kong F, Singh RP (2008) A model stomach system to investigate disintegration kinetics of solid foods during gastric digestion. J Food Sci 73:202-210

Kong F, Singh RP (2009a) Digestion of raw and roasted almonds in simulated gastric environment. Food Biophysics 4:365-377

Kong F, Singh RP (2009b) Modes of disintegration of solid foods in simulated gastric environment. Food Biophysics 4:180-190

Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H, Oberdorster G, Ziesenis A (2002) Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. J Toxicol Environ Health 65:A1513-1530

Langer R (1990) New methods of drug delivery. Science 249:1527-1533

Levenspiel O (1999) Chemical reaction engineering. Wiley, New York

Lin CC, Metters AT (2006) Hydrogels in controlled release formulations: Network design and mathematical modeling. Adv Drug Deliv Rev 58:1379-1408

Lucas P (2004) Dental functional morphology. Cambridge University Press, Cambridge

Macheras P, Argyrakis P (1997) Gastrointestinal drug absorption: Is it time to consider heterogeneity as well as homogeneity? Pharm Res 14:842-847

Mandalari G, Faulks RM, Rich GT, Lo Turco V, Picout DR, Lo Curto RB, Bisignano G, Dugo P, Dugo G, Waldron KW (2008) Release of protein, lipid, and vitamin E from almond seeds during digestion. J Agric Food Chem 56:3409-3416

Marciani L, Faulks R, Wickham M, Bush D, Pick B, Wright J, Cox E, Fillery-Travis A, Gowland P, Spiller R (2008) Effect of intragastric acid stability of fat emulsions on gastric emptying, plasma lipid profile and postprandial satiety. Br J Nutr 101:919-928

Marciani L, Gowland PA, Fillery-Travis A, Manoj P, Wright J, Smith A, Young P, Moore R, Spiller RC (2001) Assessment of antral grinding of a model solid meal with echo-planar imaging. Am J Physiol 280:G844-849

McClements DJ, Decker EA, Park Y, Weiss J (2008) Designing food structure to control stability, digestion, release and absorption of lipophilic food components. Food Biophysics 3:219-228

Michaelis L, Menten ML (1913) Die kinetik der invertinwirkung. Biochem Z 49:333-369

Moretti D, Zimmermann M, Wegmuller R, Walczyk T, Zeder C, Hurrell R (2006) Iron status and food matrix strongly affect the relative bioavailability of ferric pyrophosphate in humans. Am J Clin Nutr 83:632

Müller RH, Böhm BHL, Grau MJ (1999) Nanosuspensionen: Formulierungen für schwerlösliche arzneistoffe mit geringer bioverfügbarkeit. 2. Mitteilung: Stabilität, biopharmazeutische aspekte, mögliche arzneiformen und zulassungsfragen. Pharmazeutische Industrie 61:175-178

Nel A, Xia T, Madler L, Li N (2006) Toxic potential of materials at the nanolevel. Science 311:622-627

Nernst W (1904) Theorie der reaktionsgeschwindigkeit in heterogenen systemen. Z Phys Chem 47:52-55

Nielsen EK, Ingvartsen KL (2000) Effect of cereal type, disintegration method and pelleting on stomach content, weight and ulcers and performance in growing pigs. Livestock Prod Sci 66:271-282

Norris DA, Sinko PJ (1997a) Effect of size, surface charge, and hydrophobicity on the translocation of polystyrene microspheres through gastrointestinal mucin. J Appl Polym Sci 63:1481-1492

Norris DA, Sinko PJ (1997b) The role of surface hydrophobicity in the transport of polystyrene microspheres through caco-2 cell monolayers and intestinal mucin. Proceedings of the Controlled Release Society:17–18

Nusrat A, Turner JR, Madara JL (2000) Molecular physiology and pathophysiology of tight junctions: IV. Regulation of tight junctions by extracellular stimuli: Nutrients, cytokines, and immune cells. Am J Physiol 279:G851-857

Oh DM, Curl RL, Amidon GL (1993) Estimating the fraction dose absorbed from suspensions of poorly soluble compounds in humans: A mathematical model. Pharm Res 10:264-270

Parada J, Aguilera JM (2007) Food microstructure affects the bioavailability of several nutrients. J Food Sci 72:R21-R32

Parrott N, Lave T (2008) Applications of physiologically based absorption models in drug discovery and development. Mol Pharmaceut 5:760-775

Parrott N, Lukacova V, Fraczkiewicz G, Bolger MB (2009) Predicting pharmacokinetics of drugs using physiologically based modeling - application to food effects. AAPS J 11:45-53

Pothakamury UR, Barbosa-Cánovas GV (1995) Fundamental aspects of controlled release in foods. Trends Food Sci Technol 6:397-406

Read NW, Welch IML, Austen CJ, Barnish C, Bartlett CE, Baxter AJ, Brown G, Comption ME, Hume KE, Storie I (2007) Swallowing food without chewing; a simple way to reduce postprandial glycaemia. Br J Nutr 55:43-47

Rock CL, Lovalvo JL, Emenhiser C, Ruffin MT, Flatt SW, Schwartz SJ (1998) Bioavailability of β-carotene is lower in raw than in processed carrots and spinach in women. J Nutr 128:913-916

Santus G, Baker R (1995) Osmotic drug delivery: A review of the patent literature. J Controlled Release 35:1-21

Scholz A, Kostewicz E, Abrahamsson B, Dressman JB (2003) Can the USP paddle method be used to represent in-vivo hydrodynamics? J Pharm Pharmacol 55:443-451

Singh B, Kim K (2000) Floating drug delivery systems: An approach to oral controlled drug delivery via gastric retention. J Controlled Release 63:235-259

Sinha V, Kumria R (2001) Polysaccharides in colon-specific drug delivery. Int J Pharm 224:19-38

Tornberg E (2005) Effects of heat on meat proteins - implications on structure and quality of meat products. Meat Science 70:493-508

Uhrich K, Cannizzaro S, Langer R, Shakesheff K (1999) Polymeric systems for controlled drug release. Chem Rev 99:3181-3198

Van Buggenhout S, Sila DN, Duvetter T, Van Loey A, Hendrickx M (2009) Pectins in processed fruits and vegetables: Part III - texture engineering. Compr Rev Food Sci Food Saf 8:105-117

van het Hof KH, de Boer BCJ, Tijburg L, Lucius BRHM, Zijp I, West CE, Hautvast JGAJ, Weststrate JA (2000a) Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. J Nutr 130:1189-1196

van het Hof KH, Tijburg LBM, Pietrzik K, Weststrate JA (2007) Influence of feeding different vegetables on plasma levels of carotenoids, folate and vitamin C. Effect of disruption of the vegetable matrix. Br J Nutr 82:203-212

van het Hof KH, West CE, Weststrate JA, Hautvast JGAJ (2000b) Dietary factors that affect the bioavailability of carotenoids. J Nutr 130:503-506

Vincent JFV (1990) Fracture properties of plants. Adv Bot Res 17:235–287

Volkheimer G, Schulz FH, Lehmann H, Aurich I, Hübner RH, Hübner M, Hallmayer A, Münch H, Oppermann H, Strauch S (1968) Primary portal transport of persorbed starch granules from the intestinal wall. Pharmacology 18:103-108

Wondra KJ, Hancock JD, Behnke KC, Hines RH, Stark CR (1995) Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J Anim Sci 73:757-763

Yu LX, Amidon GL (1999) A compartmental absorption and transit model for estimating oral drug absorption. Int J Pharm 186:119-125

Yu LX, Lipka E, Crison JR, Amidon GL (1996) Transport approaches to the biopharmaceutical design of oral drug delivery systems: Prediction of intestinal absorption. Adv Drug Deliv Rev 19:359-376

Zhou JR, Gugger ET, Erdman JW (1996) The crystalline form of carotenes and the food matrix in carrot root decrease the relative bioavailability of beta- and alpha-carotene in the ferret model. J Am Coll Nutr 15:84-91

Chapter 3
Physical Aspects of the Digestion of Carbohydrate Particles

Contents

3.1 Introduction

While carbohydrates, notably glycogen, are present in animal tissue, the bulk of carbohydrate in the human diet is derived from plants. Root and vegetable crops, which include potatoes, manioc, sweet potato and pumpkin, are important sources of carbohydrate, although they are consumed in lesser quantities than starchy cereals, such as rice, maize and wheat.

Plant tissues generally exhibit morphological and mechanical heterogeneity over a range of scales. The strength of the cuticle, stems and seeds at a macroscopic level (Vincent 1990), and the strength of the secondary cell wall, and lignified elements of the vascular system (Wilson and Hatfield 1997) can all influence the ease with which the tissue may be broken down during mastication and digestion. This breakdown determines the size of the particles generated relative to the size of the contained carbohydrate storage structures, i.e. the starch granules. Given that lignin, a constituent of the secondary walls of plant cells and one of the principal structural elements that impart strength, can also impart resistance to digestion (Besle et al. 1994), it follows that fracture at a cellular level may be required to render contents available for digestion (Wilson and Hatfield 1997).

With Contribution by Allan Hardacre and John Munro

R. G. Lentle, P. W. M. Janssen, *The Physical Processes of Digestion,*
DOI 10.1007/978-1-4419-9449-3_3,

Native starch is generally stored in granules in a stable, highly organised, semicrystalline form that is resistant to hydration and digestion. Its digestibility can be increased by grinding or cooking, the latter causing the granules to undergo hydration and gelatinisation. However, there is considerable variation in the size, composition, microstructure, and hence digestibility of starch granules, depending on their source.

Carbohydrates include a broad range of substances other than starch and simple sugars, notably gums and celluloses. Non-starch polysaccharides (NSP), including pectin, inulin, hemicellulose and food gums (guar, locust bean, gum arabic, agar), resist digestion in the foregut where they can influence the rheology of digesta and compromise mixing (see Chap. 6). These substances may undergo microbial digestion in the hindgut, sometimes generating beneficial metabolites such as butyric acid, which has sparked a renewed interest in the mechanics of their digestion.

In view of their importance, we shall first consider the dynamics of the synthesis as well the molecular and microscopic organisation of starch granules before moving on to examine the effects of cellular organisation and gross morphology on the bioavailability of the contained starch. We will also touch briefly on the methods of improving this bioavailability. Finally, we will discuss the digestion of other forms of carbohydrate including glycogen in meat.

3.2 Starch

Starch is a very accessible form of energy, and plants have evolved special measures to protect their stores from opportunist consumers, including animals, fungi and bacteria, and from the effects of existing in a hydrating entropic environment. Thus starch is stored in a stable, organised, semicrystalline, and environmentally protected form until it is required by the plant. Before animals can use native starch as a dietary energy source, they have to overcome the steps the plant has taken to protect its energy reserves. There are three major barriers that can limit the rate of starch digestion: the protective structures of the plant, e.g. cell walls; the barrier at the granule surface; and lastly, the obstructive molecular packing of starch polymers within the starch granule. These microstructural barriers must be overcome to enable the enzymatic depolymerisation of starch molecules to sugars, including glucose, maltose and other dextrins.

This section is principally concerned with the influence of the physical form of the carbohydrate present as particles in the digesta on the process of human enzymatic digestion. In the context of plant starch granules, it is important to understand their molecular and microscopic structure as this has an important bearing on their digestibility. This structure is a consequence of the manner in which they are synthesised.

3.2.1 Composition and Molecular Structure of Starch

Starch is formed in the chloroplasts of green leaves during periods of light, but the amount present diminishes during darkness. In the storage tissue of plants, starch is

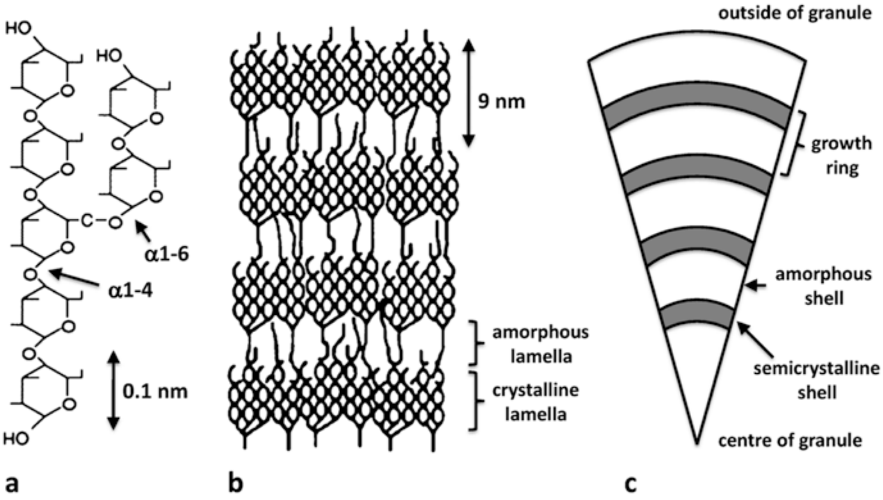


Fig. 3.1 Starch structure showing: **a** glucose chains and branching; **b** lamellar microstructure of starch granules; **c** growth rings corresponding to diurnal fluctuations

synthesised principally in non-pigmented amyloplasts. The rate of starch accumulation in the amyloplasts varies diurnally, with season and with the age of the storage tissue. Amyloplast structure may remain relatively intact at maturity, such as in legumes, or be largely lost as occurs in the principal cereal crops. Soluble glucose is transported from the chloroplasts to the amyloplasts, where it is converted into starch by the action of a suite of enzymes, probably in three stages. Initially, glucose molecules are successively linked with α1-4 glycosidic bonds to form short chains. These 'proto-starch' molecules are subsequently elongated by addition of further glucose moieties linked by α1-4 glycosidic bonds and or α1-6 glycosidic bonds (Fig. 3.1a).

Starch consists of two polymers: amylose and amylopectin. Amylose comprises more or less unbranched linear α1-4 linked chains of glucose with a molecular weight of about 0.5 million and a degree of polymerisation of between 1,500 and 6,000 the range depending on the plant species. Amylopectin is branched, containing significant numbers of α1-6 linkages at intervals on the α1-4 linked primary chains. Amylopectin has a molecular weight of 50–500 million and a degree of polymerisation in the millions, depending on the plant species (Thomas and Atwell 1999). The ratio of amylose to amylopectin is remarkably similar in the storage organs of many plants with amylopectin contents in the range 70–80% and amylose contents 20–30% of the granule (Eliasson 2006). It is possible that this ratio represents the best compromise between storage stability and ease with which starch can be mobilised. However, some naturally occurring plant variants have amylose to amylopectin ratios markedly different from the typical 1:3 value. Thus in the 'waxy' variants of maize and potato, about 99% of the starch is stored as amylopectin while in the Hi-maize® variant, up to 70% of the starch is stored as amylose.

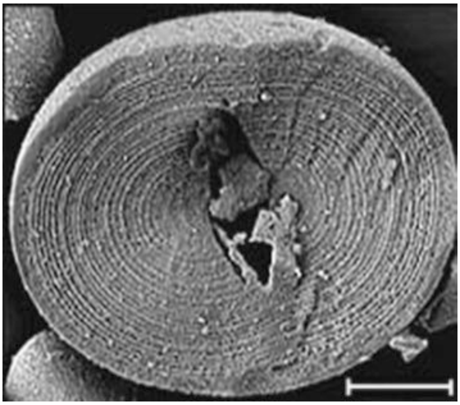


Fig. 3.2 Transmission electron microscope (TEM) showing the growth rings in a fractured potato starch granule. Scale bar 5 µm. (Pilling and Smith 2003)

The growth of the starch molecules during synthesis is thought to proceed radially from the non-reducing ends of the molecule, which are located on the surface of the starch granule. Hence the granules progressively increase in diameter as short polysaccharide chains are successively attached. At a high resolution, the granule structure consists of concentric lamellae of alternating amorphous and crystalline starch with a 9–10-nm periodicity (Fig. 3.1b). Amylose is arranged as randomly dispersed radially arranged chains among the amylopectin molecules (Jane et al. 1992; Kasemsuwan and Jane 1994). The crystalline regions have a highly organised molecular structure (Gallant et al. 1997; Ratnayake and Jackson 2007; Waigh et al. 2000) comprising of series of closely packed and aligned short branches of approximately 10–20 glucose units linked to a 'backbone' starch molecule. At a lower resolution, 'growth rings' appear as a succession of concentric shells with thicknesses of 100–400 nm (Fig. 3.2). The growth rings consist of alternating amorphous and semicrystalline shells, the latter incorporating both amorphous and crystalline lamellae (Fig. 3.1c). In part, growth rings correspond with diurnal fluctuations in the availability of sugars from photosynthesis (Smith 2001), the amorphous regions being laid down during periods when glucose is less available. The starch in the semicrystalline regions of the granule is more resistant to hydration and digestion by α-amylase than that in the amorphous regions.

In addition to the layered structure discussed above, more or less spherical 'blocklets' with diameters of 50–500 nm have been observed (Ayoub et al. 2006; Gallant et al. 1997) and are thought to have been formed during synthesis of the granule. These blocklets have a greater level of crystalline organisation, which makes them slightly more resistant to digestion by exogenous α-amylase. Indeed, the blocklet structure is best revealed on scanning electron microscope (SEM) or atomic force microscopy after digestion with α-amylase.

Proteins and lipids are often associated with the starch granules (Gallant et al., 1997) although the amounts are small, ranging from 0.1 to 0.8% of dry weight for lipid and 0.06 to 0.4% for protein (Debet and Gidley 2006). A significant proportion of proteins are found surrounding the starch granules within seeds where they may serve to sequester and protect them, and form the morphologically distinct region, the endosperm, which is evident in hard grain types such as rice, maize and milling wheat. Another protein is dispersed within the starch granule (Baldwin 2001; Debet and Gidley 2006) and in maize is thought to comprise the remnants of granule-bound starch synthetase (GBSS), a residue from the period of starch synthesis (Han et al. 2005). A number of lipids are also found in association with amylose molecules (Debet and Gidley 2006; Morrison 1995), but their function is not known. However, it has been postulated that the protein and lipids that are associated with the surfaces of starch granules may function together to modify the rate at which the granules take up water (Debet and Gidley 2006).

3.2.2 Microstructure of Starch Granules and Their Disposition in Plants

The size of starch granules varies between plant species. Starch granules in the main cereal crops range in size from 5 to 35 µm while those of root crops are somewhat larger, ranging from 4 to 40 µm in sweet potato to 22–85 µm in arrowroot. The smallest starch structures are the 2–5-µm-diameter 'sub-granules' found in rice (Satin 1998).

The surfaces of many types of starch granule have been shown to contain pores that communicate with the hilar region near the centre of the starch granule. The extents to which these pores are developed vary between plants. They can be well developed in maize but are smaller and more dispersed in potato and tapioca (Juszczak et al. 2003). The function of these pores is not well understood but may facilitate the passage of cytoplasmic fluids into the centre of the granules (Copeland et al. 2009). The pore structure may also facilitate the leaching out of amylose and subsequent gelatinisation during cooking (Gallant et al. 1997), as well as promoting permeation by digestive enzymes and the subsequent leaching of sugars (Gallant et al. 1997; Huber and BeMiller 2000; Oates 1997; Tester and Morrison 1990).

In most cereals, the amyloplast and cell wall membranes are lost or become dysfunctional as the endosperm matures. As a result, the mature endosperm comprises a series of polygonal bodies that are the residues of the original cell walls (Black 2001; French 1984; Watson 1987). In species with hard endosperms, including variants of maize (Fig. 3.3a), wheat and rice, the starch particles are embedded along with numerous protein bodies in a thin continuous proteinaceous phase (Black 2001; Watson 1987). In soft endosperm variants of maize (Fig. 3.3b) and wheat, and cereals such as oats, rye and sorghum (Earp et al. 2004), the starch granules are more rounded, and there are many voids within the continuous proteinaceous phase. The amount of protein in the endosperm does not vary significantly between plant varieties with hard and soft endosperm, but the ratio of the protein bodies to the continuous phase protein does. The behaviour of the starch granules in the hard and soft endosperm variants differs on cooking and during digestion, the granules in the hard endosperm variants being much slower to hydrate as a result of the adhesive properties of the protein matrix, which causes them to adhere together in

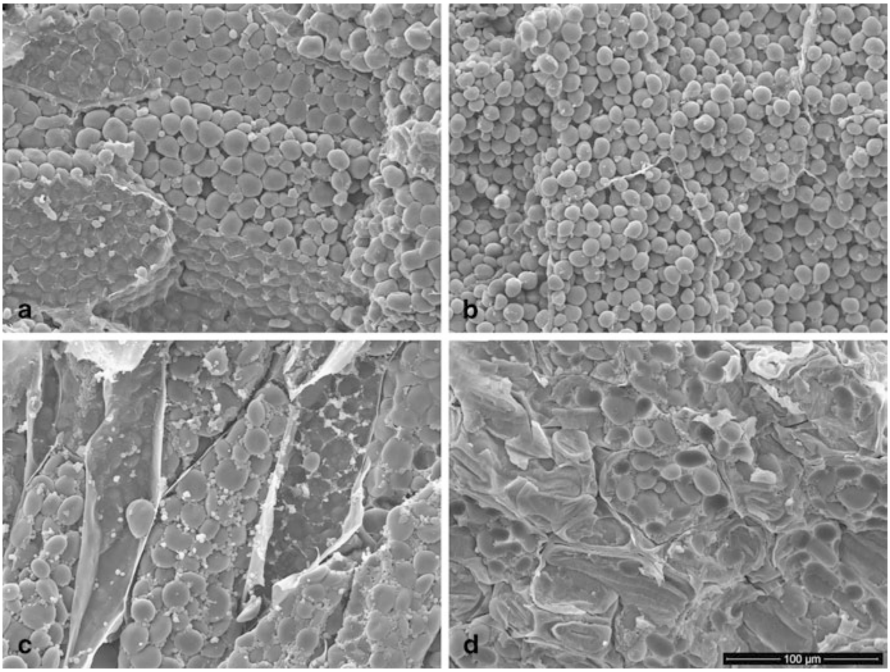


Fig. 3.3 SEM of the fractured surfaces of plant tissues containing starch granules. **a** Hard endosperm region of a mature maize *(Zea mays)* kernel showing cell wall remnants and the polygonal starch granules embedded in a hard dense proteinaceous matrix. **b** Soft endosperm region of a mature maize *(Zea mays)* kernel showing cell wall remnants and the rounded starch granules loosely embedded in the weak proteinaceous matrix. **c** Mature barley *(Hordeum vulgare)* grain showing cell wall remnants, and the large and small disk-like starch granules loosely embedded in a granular proteinaceous matrix. **d** Mature cotyledon of a dry tick bean *(Vicia faber)* seed showing the living cells, well-defined cell walls and starch granules embedded in the cell contents. (All images have been provided by Doug Hopcroft)

large aggregates (Oates 1997). Conversely, the many voids in the soft endosperm varieties expose the contained starch granules and provide greater access for water or digestive liquids, allowing them to hydrate more quickly.

In root crops such as potato and in legumes such as beans (Fig. 3.3d), the starch granules are contained in assemblages of cells bounded by thickened cell walls. Hence in situations where the cellular structure remains intact during transit through the gut, the soluble and insoluble non-digestible polysaccharide components of these walls may impair digestion of contained starch granules. This may lead to a significant proportion of dietary starch entering the colon where it is fermented by microbial action with the production of significant amounts of gas. This phenomenon may be averted either by cooking or by fine milling, which disrupt the cellular elements and facilitate the entry of digestive enzymes. The cell walls of potato and the other starchy tubers are relatively weak and may be readily disrupted mechanically to release the starch granules. Similarly, the high water content of the cells and their permeability to water allows for the rapid and complete disruption of the cellular structure by cooking and may facilitate the gelatinisation of contained starch granules.

In most grain legumes, with the exceptions of fresh peas and beans, the cell walls are thick, and the living endosperm is dry and resistant to hydration. Hence the digestibility of contained granules is very dependent on the method of cooking or processing (Kozlowska 2001).

3.2.3 Digestion of Starch

When a starchy food is ingested, the starch may be either in its native state or in a gelatinised form, depending on the level of processing that the food containing it has received. It is then masticated, mixed with salivary α-amylase, and formed into a 'swallow-safe' bolus (see Chap. 8). Salivary α-amylase, like pancreatic α-amylase, is an endoamylase. It has an optimum pH of 6.7–7.0 but may remain active in the stomach for some time due to the persistence of boluses and the alkaline conditions within them (Marciani et al. 2001). The sieving action of the stomach (see Chap. 9) separates the starchy slurry from larger food particles and allows it to transit to the small intestine where it is admixed with pancreatic α-amylase. The latter, like other α-amylases, can hydrolyse the α-1,4 glycosidic bonds in the amylose and amylopectin molecules, but cannot cleave the α-1,6 links at branch points in the amylopectin. Hence the product of enzymatic digestion of starch in the lumen is a mixture of maltose, maltotriose and α-limit dextrins. These products must transit the lumen and mucus layers to the intestinal wall. In the region close to the enterocytes, which can be regarded as a subsidiary compartment beneath the mucus layer, the depolymerisation of starch to glucose is completed by a series of oligosaccharidases that include α-sucrase, α-dextrinase, α-glucoamylase, trehalase, lactase and β-galactosidase. These enzymes have a characteristic hydrophobic amino acid sequence at either the amino- or carboxyl-terminus, which promotes their anchoring in the plasma membrane of the enterocyte brush border (Stipanuk 2000). When the tips of enterocyte microvilli are 'pinched off' to form vesicles, these enzymes remain associated with the latter (see Chap. 10), an association that may act to limit their action to the compartment beneath the mucus layer. The glucose produced by their action can then be absorbed by adjacent enterocytes via specific membrane-bound glucose transport proteins, notably sodium-glucose-linked transporter (SGLT) and glucose transporter (GLUT) transporters (see Chap. 10).

Three major physical barriers can limit the rate at which starch is digested: the extracellular structures of the plant, e.g. cell walls; the barrier at the surface of the granule; and the molecular packing of starch polymers within the starch granule. These barriers may be disrupted by external mechanical disruption, by cooking, by chewing, and by the shearing and abrading action of intestinal contractions on digesta. Thus the rate at which starch is digested and absorbed depends partly on the rate of gastric emptying (Darwiche et al. 2001), but also on the physical form in which it arrives in the small intestine. More specifically, the rates of digestion of intact starch granules, disorganised or dispersed gelatinised starch, and re-aggregated gelatinised starch, i.e. retrograded starch, differ significantly.

Even in processed food, a small proportion of the starch remains resistant to enzymatic digestion, notably that which comprises the surface of the starch granules or is trapped within the cellular structure of the tissue. This resistant starch may still be fermentable in the colon. The relative proportions of starch that are variously resistant to digestion can be assessed in starches from different sources by determining their rate of enzymatic digestion in vitro (Englyst et al. 1992), i.e. the fractions of starch that are rapidly digested (RDS; 0–20 min), slowly digested (SDS; 20–120 min), and digestion resistant (RS). The RS fraction can be further classified based on the mechanism of its resistance.

3.2.3.1 The Digestion of Native Starch Granules

Native starch granules are digested relatively slowly over a period of hours, the morphological sequence of breakdown varying with the species of plant from which the granules originate (Donald 2004; Oates 1997). The initial stage involves the erosion of the granule surface and subsequent penetration of the broken surface by the formation of pits, furrows or (more usually) by the enlargement of natural pores (Fig. 3.4). This allows amylases to access the interior (Han and Hamaker 2002; Oates 1997). These enzymes rapidly digest the amorphous regions of the granule and subsequently digest the ordered or semicrystalline regions, so that the interior of the granule is completely digested leaving only a shell of the granule's surface, and other more resistant fragments of starch.

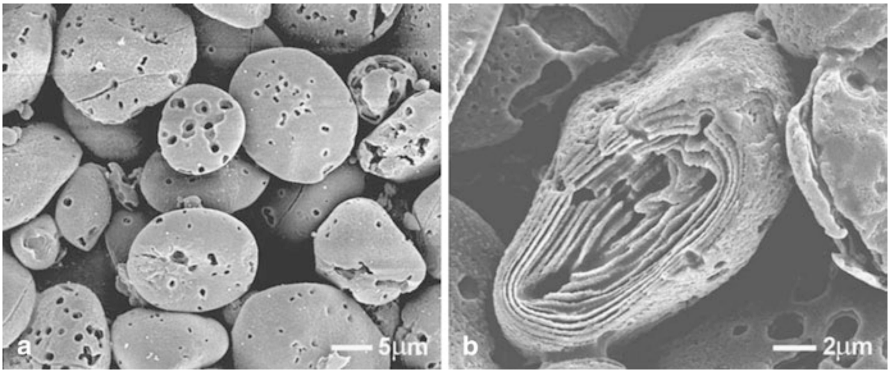


Fig. 3.4 SEM images of waxy hull-less barley starch following 1 h of in vitro digestion with porcine pancreatic α-amylase. Note the pitting and the annular channels giving amylase access to the granule interior. Compare the resistance of outer surface and semicrystalline growth rings with the extensive digestion of the amorphous regions within the granule. (Li et al. 2004)

3.2.3.2 Effect of Cooking on the Digestion of Starch

Gelatinisation Seeds and their contained starches are generally cooked prior to consumption by humans. Heating native starch granules in the presence of excess water causes the starch to gelatinise, an effect that dramatically increases its digestibility and the rate at which it can be digested. The native granules of some plants, such as the potato, are virtually indigestible, but they can be rapidly and completely digested once gelatinised (Fig. 3.5). Progressive hydration during the process of gelatinisation causes the granules to swell to many times their original volume and causes amylose to leach from the granule into the surrounding aqueous phase. At the same time, the tightly packed semicrystalline structure of the starch chains within the granule is disrupted, along with any ability of this structure to sterically exclude digestive enzymes (Donald 2004). The form of the original granules may be preserved after swelling but is considerably weakened and can be readily disrupted by the application of shear.

When starch granules are sequestered in cells with robust cell walls, such as those in legumes, there is generally sufficient moisture present for the granules to gelatinise in situ, although the temperatures required to induce this are somewhat higher (Aguilera et al. 2001). Such in situ swelling can cause the granules to exert significant pressure on the walls of the containing cells, which may help to weaken intercellular adhesion (Jarvis et al. 1992). Such disruption is important as the integrity of cell walls in legumes is known to constitute a significant barrier to digestion (Tovar et al. 1990; Tovar et al. 1992).

Differences in the relative proportions of lipids, proteins or amylose in starch granules are known to influence their behaviour during processing. On this basis, granules have been broadly grouped into three categories: those that swell rapidly during processing (group 1), e.g. potato and waxy starches; those that exhibit slow

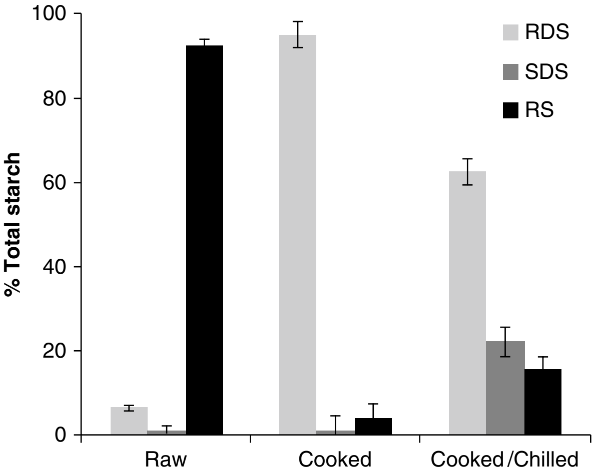


Fig. 3.5 Effect of cooking on the in vitro digestibility of starch in potato. The digestion was performed on raw potato, freshly cooked potato and potato that was cooked and chilled for 2 days to induce retrogradation (*RDS* rapidly digested starch; *SDS* slowly digested starch; *RS* resistant starch)

swelling above gelatinisation temperature (group 2), e.g. 'normal' wheat and maize; and those that display highly restricted swelling (group 3) (Debet and Gidley 2006). Group 2 granules have a greater proportion of lipid and protein on their surfaces than granules from group 1. Moreover, the ease and degree of swelling of group 2 granules increase after the lipids and proteins have been removed by washing with sodium dodecyl sulphate solution. Hence these components may help to prevent the ingress of water and digestive enzymes, and contribute to the small proportion (5–15%) of starch classified as resistant when the in vitro digestibility of isolated and gelatinised normal starch granules is measured. Group 3 granules include those with high amylose content that are found in some varieties of maize and potato, and which do not swell significantly in water at temperatures below 100 °C. The resistance to swelling probably results from the close packing of the amylose molecules within the granule. The high amylose maize starches are not easily digested after cooking owing to the high temperatures that are required to gelatinize them (> 110 °C), temperatures that are above those normally used to cook the water-rich foods that contain them.

Retrogradation Gelatinised starch can undergo retrogradation upon cooling and storage, a process in which dispersed molecular starch chains re-associate into new enzyme-resistant conformations. Retrogradation appears to occur at different rates in the two starch polymer fractions; it occurs rapidly with the amylose fraction but takes several days with amylopectin. Hence the rate and extent of retrogradation that occurs in a particular food depends on the amount of amylose present.

It is possible to enzymatically digest retrograded starch but the speed depends on the degree of retrogradation and on the strength of association of the starch chains. For example, the overall digestibility of potato starch varies considerably with form. The bulk of raw granular potato starch is categorised as resistant but most of this starch is rapidly digestible after cooking to the point of gelatinisation. However, the portion of rapidly digestible starch is reduced, and that of slowly digestible and resistant starch is increased, after cooling to induce retrogradation (Fig. 3.5).

Other Heat Treatments While the usual aim of food processing is to induce gelatinisation, two other heat treatment processes are also used on occasion to improve digestibility, annealing and heat moisture treatment (HMT). Annealing involves the heating of starch granules, along with excess water, to a temperature below the gelatinisation temperature and maintaining it long enough to allow the starch to undergo molecular reorganisation. HMT involves heating starch granules to a high temperature (100–120 °C) under condition where moisture is restricted (< 30%), which again brings about molecular reorganisation. While the microstructural changes induced by annealing and HMT are well defined, their effect on enzymatic hydrolysis is somewhat variable (Chung et al. 2009).

The availability of water during cooking can substantially modulate the effects of cooking on digestibility. Where the water content of food products is not sufficient to allow the contained starch granules to become fully gelatinised, e.g. dry rolled oats in the form of muesli, the digestibility is reduced. Where food products contain other hydrocolloids in addition to starch, such as the NSP in remnants of cell walls, or intrinsic and added gums or sugars, then competition for water may again reduce the extent to which starch is gelatinised during cooking (Pomeranz et al. 1977).

3.2.3.3 Effect of Milling and Particle Size on Digestion

A number of physical food processing operations may impact upon the digestion of carbohydrate containing particles derived from plants. Fragmentation promotes the ingress of digestive enzymes by physically breaching many of the morphological barriers and can liberate component structures. The success of such fragmentation depends upon the scale of barriers in relation to the size of the particles that are generated. Milling readily removes the large protective structures that surround the starchy endosperm component of the seed and may also disrupt tough cellular structures, which can protect starch granules from digestion in the small intestine. However, the success of the latter depends upon the size of the starch granules in relation to the fineness of the particles that are generated. If the size of the particles is sufficiently small to enable the walls of individual cells to be breached, then the contained starch can be quickly degraded, provided it has been gelatinised (Hallfrisch and Behall 2000). Thus grinding to a finer particle size causes the RDS fraction to increase and the RS fraction to decrease accordingly (Fig. 3.6). Grinding seeds with a very soft endosperm, such as barley, oats and some maize varieties, cannot only liberate individual starch granules, but very aggressive processing can crack and damage the starch granules themselves (Donald 2004).

Chewing may increase the digestibility of food that contains intact grains or large grain fragments, such as rice, wheat or maize, and food that contains dense,

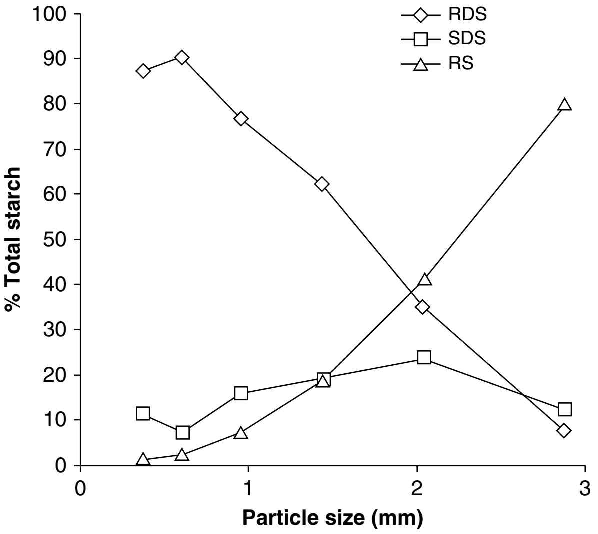


Fig. 3.6 In vitro digestion of cooked milled wheat of different particle sizes. Wheat of larger particle size contains a greater proportion of *resistant starch (RS)* a lower proportion of rapidly digested starch *(RDS)* than those of finer particle sizes. There is little change in slowly digested starch *(SDS)* with particle size

non-porous starch matrices that limit enzyme penetration so that digestion is largely confined to the surface, such as in pasta. The digestibility of many processed foods is not improved by chewing as they are based on ingredients, such as finely milled flour, that have been previously cooked and formed into a weak, hydrated structure in which most, if not all the starch is gelatinised.

3.3 Glycogen

Small quantities of carbohydrate are stored in animal tissue as glycogen, which accounts for 1–2% of the fresh weight of muscle and is particularly concentrated in the liver (up to 8%). Like amylopectin, glycogen is a polymer of D-glucose but the ratio of branched α1-6 linkages to linear α1-4 linkages is twice that of amylopectin. Thus glycogen is susceptible to hydrolysis by the same enzymes as the starch polymers. Studies suggest that purified animal glycogen is digested at a similar rate to that of purified short chain rice starches (Azad and Lebenthal 1990). However, as in the case of plant starches, the rate of digestion may be lower when glycogen granules are contained in intact tissue owing to the physical effects of their structural organisation.

The morphology of glycogen in muscle, liver and other tissues is similar to that of starch in plants. Glycogen occurs in spheroidal 'beta granules' of 20–30 μm in diameter, which are grouped into larger rosettes, termed alpha granules (Drochmans 1962). Each beta granule is composed of a number of identical subunits, termed gamma particles, which are each around 3 μm in diameter (Drochmans 1962). Particulate glycogen is considered by some to be a dynamic organelle (Scott and Still 1968). The protein constituents of beta granules include a glycogen-synthesising component which is associated with the cellular endoplasmic reticulum and contains a number of enzymes including glycogenin, glycogen synthase, phosphorylase kinase and phosphatase (Roach 2002; Roach et al. 1998).

3.4 Colonic Fermentation

A broad range of carbohydrates, including resistant starches, non-starch polysaccharides, oligosaccharides and mucins, reach the large intestine where they undergo microbial fermentation that produces an array of short chain fatty acids (SCFA), mainly acetate, propionate, and butyrate. The rates of production and quantities of the various SCFA produced depends on the range of microbial species and the numbers of each species, the substrate type and the gut transit time (Topping and Clifton 2001). It has been suggested that the extent of digestion in the small intestine is optimised to allow 10–15% of the starch consumed, the so-called resistant starch fraction, to spill over into the colon to maintain the colonic flora. It has been estimated that SCFA typically contribute 10% of the energy requirement for humans and 20–30% for several other hindgut-fermenting animals (Bergman 1990).

Fermentation appears to occur predominantly in the proximal colon, while the SCFA are absorbed progressively along the large bowel (Topping and Clifton 2001). Carbohydrates may enter the colon in solution in the liquid phase of digesta, as colloidal dispersions, and in larger particulate fractions such as those from cell walls. The dynamics that govern their digestion are complex. To achieve cellulolysis, bacteria need first to adhere to the substrate (Flint et al. 2008), and hence the bacteria must be well dispersed through the incoming particulate material in the more fluid digesta of the proximal colon. Most plant matter contains a surface layer that is resistant to attachment of degradative microflora and therefore is resistant to digestion. Further, the digestibility of plant cells may be restricted by the anatomical structure of the cellular material, notably the presence of lignified secondary cells walls (Wilson and Hatfield 1997). Breakdown of the plant matter into constituent particles allows saccharolytic and cellulolytic microflora to attach to and enzymatically digest any substrate remaining after digestion in the small intestine. Microorganisms that primarily colonise and become firmly attached to the substrate are recruited from a pool of unattached organisms in the surrounding fluid phase (Czerkawski and Cheng 1988). The glycocalyceal-enclosed colonies subsequently form trophic associations with secondary colonising species (Latham 1980) and become organised into an adherent biofilm of ‘digestive consortia’, which are less readily inhibited or broken down by substances such as tannins and certain soluble non-starch polysaccharides, e.g. methyl cellulose (McAllister et al. 1994).

Similar dynamics may cause consortia of bacteria also to adhere to the mucus layer of the intestinal wall (Deplancke and Gaskins 2001). Some members of the trophically organised species that adhere to the mucus layer of the host appear to engage in ‘cross-talk’ with, and modulate the function of, various elements of the host’s immune system (Kelly et al. 2003). The extents to which cellulolytic members of this mucus-adherent self-replicating (autochthonous) community exfoliate and are dispersed among incoming sources of cellulose are not known, nor are the dynamics of their dispersal. Indeed, it is possible that those cellulolytic microflora that are part of a more transient particle-adherent non-self-replicating (allochthonous) lumen dwelling community are more likely to adhere.

The mass transfer of the products of cellulolysis, i.e. SCFA, from the lumen to the epithelial surface and their subsequent absorption are dealt with in detail in Chap. 6.

3.5 Future Directions

One important current challenge for human nutritionists and food technologists is to develop processes that control the physical characteristics of starch granules and associated plant structures in such a way as to restrict starch digestion, and tailor the rate of liberation and absorption of glucose to be compatible with the physiological dynamics of the consumer. Further, to provide a degree of particulate heterogeneity that is sufficient to ensure a proportion of undigested starch or NSP reaches the colon and is available for fermentative digestion, thereby maintaining a healthy population of cellulolytic microflora and an appropriate yield of SCFA in the large intestine.

References

Aguilera JM, Cadoche L, Lopez C, Gutierrez G (2001) Microstructural changes of potato cells and starch granules heated in oil. Food Res Int 34:939-947

Ayoub A, Ohtani T, Sugiyama S (2006) Atomic force microscopy investigation of disorder process on rice starch granule surface. Starch-Starke 58:475-479

Azad MAK, Lebenthal E (1990) Role of rat intestinal glucoamylase in glucose polymer hydrolysis and absorption. Pediatr Res 28:166-170

Baldwin PM (2001) Starch granule associated proteins and polypeptides : A review. Starch/Staerke 53:475-503

Bergman EN (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev 70:567-590

Besle JM, Cornu A, Jouany JP (1994) Roles of structural phenylpropanoids in forage cell wall digestion. J Sci Food Agric 64:171-190

Black JL (2001) Quality feed grains: Research highlights and opportunities. 10th Australian barley technical symposium, Canberra

Chung HJ, Liu Q, Hoover R (2009) Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. Carbohydr Polym 75:436-447

Copeland L, Blazek J, Salman H, Tang MCM (2009) Form and functionality of starch. Food Hydrocolloids 23:1527-1534

Czerkawski JW, Cheng KJ (1988) Compartmentation in the rumen. In: Hobson PN (ed) The rumen microbial ecosystem. Elsevier, Barking, pp 361-386

Darwiche G, Ostman EM, Liljeberg HGM, Kallinen N, Bjorgell O, Bjorck IME, Almer LO (2001) Measurements of the gastric emptying rate by use of ultrasonography: Studies in humans using bread with added sodium propionate. Am J Clin Nutr 74:254-258

Debet MR, Gidley MJ (2006) Three classes of starch granule swelling: Influence of surface proteins and lipids. Carbohydr Polym 64:452-465

Deplancke B, Gaskins H (2001) Microbial modulation of innate defense: Goblet cells and the intestinal mucus layer. Am J Clin Nutr 73:1131S

Donald AM (2004) Understanding starch structure and functionality. In: Eliasson AC (ed) Starch in food: Structure, function and applications. Woodhead, Cambridge, pp 156-184

Drochmans P (1962) Morphologie du glycogène: Etude au microscope électronique de colorations négatives du glycogène particulaire. J Ultrastruct Res 6:141-163

Earp CF, McDonough CM, Rooney LW (2004) Microscopy of pericarp development in the caryopsis of sorghum bicolor (l.) Moench. J Cereal Sci 39:21-27

Eliasson A-C (2006) Starch: Physiochemical and functional aspects. In: Eliasson A-C, Gudmunsson M (eds) Carbohydrates in food. CRC, Boca Raton, pp 391-470

Englyst HN, Kingman SM, Cummings JH (1992) Classification and measurement of nutritionally important starch fractions. Eur J Clin Nutr 46:S33-50

Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA (2008) Polysaccharide utilization by gut bacteria: Potential for new insights from genomic analysis. Nature Rev Microb 6:121-131

French D (1984) Organization of starch granules. In: Whistler RL, BeMiller JN, Paschall EF (eds) Starch: Chemistry and technology. Academic Press, Orlando, pp 183-247

Gallant DJ, Bouchet B, Baldwin PM (1997) Microscopy of starch: Evidence of a new level of granule organization. Carbohydr Polym 32:177-191

Hallfrisch J, Behall KM (2000) Mechanisms of the effects of grain on insulin and glucose response J Amer Coll Nutr 19:320S-325S

Han XZ, Benmoussa M, Gray JA, BeMiller JN, Hamaker BR (2005) Detection of proteins in starch granule channels. Cereal Chem 82:351-355

Han XZ, Hamaker BR (2002) Association of starch granule proteins with starch ghosts and remnants revealed by confocal laser scanning microscopy. Cereal Chem 79:892-896

Huber KC, BeMiller JN (2000) Channels of maize and sorghum starch granules. Carbohydr Polym 41:269-276

Jane J, Xu A, Radosavljevic M, Seib PA (1992) Location of amylose in normal starch granules. I: Susceptibility of amylose and amylopectin to cross-linking reagents. Cereal Chem 69:405-409

Jarvis MC, Mackenzie E, Duncan HJ (1992) The textural analysis of cooked potato. 2. Swelling pressure of starch during gelatinisation. Potato Res 35:93-102

Juszczak L, Fortuna T, Krok F (2003) Non-contact atomic force microscopy of starch granules surface. Part i. Potato and tapioca starches. Starch/Staerke 55:1-7

Kasemsuwan T, Jane J (1994) Location of amylose in normal starch granules. II. Locations of phosphodiester cross-linking revealed by phosphorus-31 nuclear magnetic resonance. Cereal Chem 71:282-286

Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AGP, Pettersson S, Conway S (2003) Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-γ and RelA. Nat Immunol 5:104-112

Kozlowska H (2001) Nutrition. In: Hedley CL (ed) Carbohydrates in grain legume seeds. CABI Publishing, Oxford,UK, pp 61-87

Latham MJ (1980) Adhesion of rumen bacteria to plant cell walls. In: Berkeley RCW, Lynch JM, Melling J (eds) Microbial adhesion to surfaces. Ellis Horwood, Chichester, pp 339–350

Li JH, Vasanthan T, Hoover R, Rossnagel BG (2004) Starch from hull-less barley: V. In-vitro susceptibility of waxy, normal, and high-amylose starches towards hydrolysis by alpha-amylases and amyloglucosidase. Food Chemistry 84:621-632

Marciani L, Gowland PA, Spiller RC, Manoj P, Moore RJ, Young P, Fillery-Travis AJ (2001) Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. Am J Physiol 280:G1227-1233

McAllister TA, Bae HD, Jones GA, Cheng KJ (1994) Microbial attachment and feed digestion in the rumen. J Anim Sci 72:3004-3018

Morrison WR (1995) Starch lipids and how they relate to starch granule structure and functionality. Cereal Foods World 40:437-446

Oates CG (1997) Towards an understanding of starch granule structure and hydrolysis. Trends in Food Science and Technology 8:375-382

Pilling E, Smith AM (2003) Growth ring formation in the starch granules of potato tubers. Plant Physiol 132:365-371

Pomeranz Y, Shogren MD, Finney KF (1977) Fiber in breadmaking – effects on functional properties. Cereal Chem 54:25-41

Ratnayake WS, Jackson DS (2007) A new insight into the gelatinization process of native starches. Carbohydr Polym 67:511-529

Roach PJ (2002) Glycogen and its metabolism. Curr Mol Med 2:101-120

Roach PJ, Cheng C, Huang D, Lin A, Mu J, Skurat AV, Wilson W, Zhai L (1998) Novel aspects of the regulation of glycogen storage. J Basic Clin Physiol Pharmacol 9:139-151

Satin M (1998) Functional properties of starches. FAO report

Scott RB, Still WJS (1968) Glycogen in human peripheral blood leukocytes: II. The macromolecular state of leukocyte glycogen. J Clin Invest 47:353-359

Smith AM (2001) The biosynthesis of starch granules. Biomacromolecules 2:335-341

Stipanuk MH (2000) Biochemical and physiological aspects of human nutrition. Saunders, Philadelphia

Tester RF, Morrison WR (1990) Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. Cereal Chem 67:551-557

Thomas DJ, Atwell WA (1999) Starches. Eagan Press, St Paul

Topping DL, Clifton PM (2001) Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. Physiol Rev 81:1031-1064

Tovar J, Bjoerck IM, Asp NG (1990) Analytical and nutritional implications of limited enzymic availability of starch in cooked red kidney beans. J Agric Food Chem 38:488-493

Tovar J, Granfeldt Y, Bjoerck IM (1992) Effect of processing on blood glucose and insulin responses to starch in legumes. J Agric Food Chem 40:1846-1851

Vincent J (1990) Fracture properties of plants. Adv Bot Res 17:235-287

Waigh TA, Kato KL, Donald AM, Gidley MJ, Clarke CJ, Riekel C (2000) Side-chain liquid-crystalline model for starch. Starch/Starke 52:450-460

Watson SA (1987) Structure and composition. In: Watson SA, Ramstad PE (eds) Corn: Chemistry and technology. American Association of Cereal Chemists, St. Paul

Wilson JR, Hatfield RD (1997) Structural and chemical changes of cell wall types during stem development: Consequences for fibre degradation by rumen microflora. Aust J Agric Res 48:165-180

Chapter 4
Physical Aspects of the Digestion of Protein Particles

Contents

4.1 Introduction

The digestion of proteins is generally assumed to be more efficient than that of fats and carbohydrates. However, as with carbohydrates, proteinaceous particles in digesta may exhibit a degree of heterogeneity that may influence their physical breakdown, solubilisation and digestion. Mechanical breakdown may be affected by the presence of proteinaceous structural components that have significant mechanical strength and elasticity, e.g. gluten in bread and collagen in meat. Moreover, some proteins, e.g. keratin, may entirely resist enzymatic digestion in the small intestine.

The heterogeneity of proteins occurs over a range of scales extending from the morphological to the molecular. Hence regions in the folded or pleated molecular architecture of proteins may possess hydrophilic or hydrophobic properties which facilitate association with the surrounding aqueous phase of digesta or with hydro-

---

With Contribution by Allan Hardacre

R. G. Lentle, P. W. M. Janssen, *The Physical Processes of Digestion,*
DOI 10.1007/978-1-4419-9449-3_4,

phobic regions in other molecules. Such characteristics may influence the behaviour or the protein at an oil–water interface and its susceptibility to proteolytic cleavage.

Protein-containing particles in digesta may be derived from a range of dietary items including the muscle and organs of terrestrial vertebrates and fish, the seeds and tubers of plants, dairy products and eggs. Meat products contain high proportions of protein, ranging between 74% (lean beef) and 90% (fish) dry weight which equates to around 15–20% wet weight (Strasburg et al. 2008). Hence digesta particles derived from this source are composed predominantly of protein and are mainly heterogeneous in regard to the morphological disposition of the component proteins within the particular tissue. Plants generally contain lower proportions of protein; legumes such as peas and beans may contain up to 45% protein while cereals contain somewhat less, e.g. rice (6%), wheat (12%) and oats (14%) dry weight. Hence particles derived from these sources are likely to exhibit heterogeneity with respect to a range of macronutrients and contain proteinaceous regions that are dispersed within a matrix of other macronutrients and indigestible material.

Particles derived from other protein-rich food sources exhibit a diverse range of heterogeneous characteristics. Thus the proteins in bovine milk (25% dry matter basis) may be dissolved in the aqueous phase or oriented across the oil–water interface of the contained fat emulsion (Fox 2003). Moreover, these distributions may change according to the digestive conditions. Hence casein micelles aggregate (clot) under gastric conditions (Moughan et al. 1991).

The purpose of this chapter is to provide an overview of the physical characteristics of protein-containing particles in digesta and the manner in which their characteristics influence flow and mixing of digesta and the digestion and absorption of the contained protein.

4.2 The Microstructure and Physical Properties of Proteins

The ease with which proteins may be digested is influenced by their molecular properties. The properties of proteins reflect differences at various levels of structural organisation (Damodaran 2008). Hence the extent to which the component amino acid chains of a protein are organised into a helical or a pleated sheet configuration has a significant effect on its solubility and its digestibility. Keratins, proteins with a predominant pleated sheet configuration, are poorly soluble in water and resist enzymatic digestion. Proteins with a globular tertiary conformation may possess regions with differing affinity for aqueous or hydrophobic environments and consequent susceptibility to digestion by proteases. The denaturation of this architecture by cooking may have profound effects on digestibility. Moreover, the ease with which the structure is denatured will influence the extent to which normal cooking processes render the protein susceptible to digestion.

Some foods have morphologically distinct regions that contain significant quantities of conjugated proteins. The linkage of the constituent protein with carbohydrate, lipid or inorganic moieties will also influence molecular behaviour and susceptibility to digestion. Thus glycoproteins in eggs and lipoproteins in egg yolk may influence the ease with which they are permeated by the aqueous phase during cooking or digestion. Hence the categorisation of proteins according to their morphological and functional disposition within the food gives some insight into the dynamics of their digestion. Meat proteins may be categorised into three groups: myofibrillar, sarcoplasmic and connective tissue (Strasburg et al. 2008; Tornberg 2005), the former being more readily and the latter more tardily digested.

4.2.1 Meat Proteins

Skeletal muscle (i.e. meat) proteins can be classified into three main categories based on their disposition and molecular structure (Tornberg 2005). The myofibrillar proteins constitute around 60% of the total protein content and include actin and myosin, and the muscle scaffolding fibres, i.e. titin, nebulin, desmin, vimentin and synemin. The fibrous proteins of connective tissue, namely collagen, reticulin and elastin, constitute about 10% of the total protein. The majority of the remainder comprises the globular proteins which include the soluble proteins within the sarcoplasm, such as myoglobin, and those occupying vascular spaces such as albumin and immune globulins.

The inherent heterogeneity of muscle arises from its morphological organisation. Each sarcolemma and its contained array of interdigitated actin and myosin myofibrils are enveloped in a matrix of types III, IV and V collagen fibres, the endomyceum. Each fascicular bundle of sarcolemmal fibres is enveloped in a common layer of types I and III collagen fibres, the perimyceum. Each functional bundle of perimyceum-bound fascicule, i.e. muscle, is enveloped in a layer of type I collagen fibres, the epimyceum (Bailey et al. 1979). The relative proportion of collagen fibres, notably in the perimyceum, varies according to the functional organisation of the muscle. Hence ‘pennate’ muscles formed from many short interdigitating fasciculi contain relative higher proportions of collagen than those formed from longer parallel fasciculi.

The tenderness of meat products, and hence the mechanical strength of meat particles, is closely associated with the morphology and the proportion of connective tissue that they contain (El Jabri et al. 2010; Purslow 2002). After slaughter, the connective tissue loses strength and the muscles relax as the internal structure begins to break down (Purslow 2002). The continuing metabolism of residual soluble sugars and stored glycogen, leads to the accumulation of lactic acid in the muscle tissue and a decrease in pH. Endogenous proteases bring about the partial digestion of the myofibrillar network causing the muscle to lose its initial rigour and become extensible (Strasburg et al. 2008).

4.2.2 Plant Proteins

The proteins found in seeds, fruits, roots and other storage structures constitute the principal dietary source of plant protein. Leaves, stems and other non-storage structures contain significantly lower proportions of proteins.

The storage proteins of seeds are packaged into organelle aggregates termed protein bodies. These proteins comprise two main groups the saline-soluble globulins and alcohol-soluble prolamines (Okita and Rogers 1996). The storage globulins are highly conserved with regard to their internal conformation and have similar structural properties (Marcone 1999). They can be separated into two main fractions with sedimentation coefficients of 7S and 11S, the latter tending to predominate in most legumes and in many other dicotyledonous plants (Shewry et al. 1995). The globulins are generally stored in a single vacuole within each cell whereas prolamines can be stored in a number of different subcellular compartments (Okita and Rogers 1996). Hence the prolamines in maize rice and sorghum are packaged in endoplasmic reticulum-delineated protein bodies whereas those in wheat barley and oat are stored in vacuolar compartments (Okita and Rogers 1996). Prolamines comprise a significant proportion of the proteins in most cereal seeds. They are deposited within the developing starchy endosperm and have no known function other than storage. They are generally rich in proline and glutein and are insoluble in water (Shewry et al. 1995). Hence, their low solubility in the aqueous phase of digesta and containment within the endosperm may impede their dissolution and digestion.

Tubers contain lower proportions of proteins than seeds, e.g. 3–6% of dry weight in potatoes. Unlike the storage proteins of seeds, the proteins in tubers vary considerably in their composition between plant species due to the latter's diverse botanic origins. A number of tuber proteins have other functions in addition to storage that include pest resistance and enzyme inhibition (Shewry 2003).

4.2.3 *Dairy Proteins*

Milk contains a variety of proteinaceous components in a range of different physical associations. Hence casein, the largest protein fraction (80% of total) is in a micellar suspension, the somewhat less abundant globular whey proteins (20%) are in aqueous solution while smaller quantities of lipoproteins are associated with the oil–water interface of native fat globules. The casein micelles are 20–300 nm in diameter and are voluminous, i.e. entrap fluid within them (Fig. 4.1) (Walstra 1979).

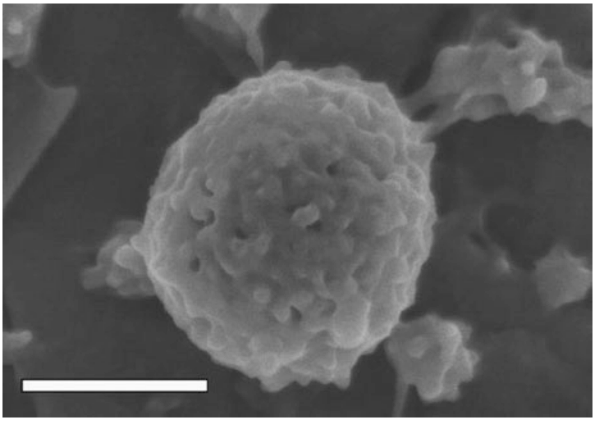


Fig. 4.1 Scanning electron microscope (SEM) of a single casein micelle. Scale bar = 200 nm. (Dalgleish et al. 2004)

Casein micelles appear to be heterogeneous in composition with different types of the protein being found at different sites. Hence κ-casein predominates on the external surface and sterically stabilises the micelle, Enzymatic hydrolysis of the stabilising layer of κ-casein or lowering the pH of the aqueous phase close to the isoelectric point (pH 4.6) may destabilise the micelles causing them to aggregate into a three-dimensional protein network as occurs in the manufacture of cheese and yogurt.

4.3 Effect of Processing on Protein Digestibility

A number of processes can influence the chemical and physical properties of proteinaceous foods and hence affect particle behaviour, the ease of permeation of digestive fluids and the process of digestion. These processes may render proteins more amenable or more resistant to digestion by endogenous enzymes. In some cases, this enables them to entirely resist enzymatic digestion in the stomach and small intestine, and undergo subsequent bacterial digestion in the large intestine (Tuohy et al. 2006). Processing may also lead to the formation of barriers that limit the permeation of digestive enzymes. Hence the browned crust of meat loaf has reduced digestibility compared to its interior (Öste and Sjödin 1984).

4.3.1 Denaturation

Denaturation of the molecular structure occurs when proteins are heated in the presence of water (cooked) to a sufficiently high temperature, at room temperature in the presence of strong acid or alkali, and at pressures between 100 MPa and 800 MPa.

Proteins are generally stable when dry. Susceptibility to denaturation increases rapidly with water content up to 35% and more slowly up to about 75%. The hydrogen bonding that occurs in aqueous systems and the links between the hydrophilic regions of the constituent amino acid chains determine the conformation of the molecule. As temperatures increase or as the pH moves away from the isoelectric point, the hydrogen bonds become increasingly unstable and the protein structure is increasingly dominated by the hydrophobic regions of the molecule causing the molecule to swell and unfold. These processes generally induce disruption of those hydrogen bonds that maintain the orderly orientation of the hydrophobic elements of the amino acid chains into specific regions within the molecule and of the hydrophilic regions toward the aqueous environment.

The conformation of a protein molecule is dominated by its minimum entropy state. At typical cooking temperatures of between 60 °C and 95 °C, this state occurs with the molecule at the maximum degree of unfolding. Hence denaturation is often irreversible (Damodaran 2008). The rearrangement of the secondary and tertiary structure, and reorientation of the constituent peptide chains can render denatured proteins more susceptible to digestion and decrease their solubility in water and salt solutions (Kauzmann 1959).

Differential scanning calorimetry has shown that in foods such as meat, egg or legume grains denaturation occurs progressively over a range of temperatures according to the thermal energy required for disruption of stabilising bonds. Hence agglutination of the globular heads of the muscle protein myosin, occurs at temperatures between 30 °C and 50 °C, and changes in the helical structures of myosin tails occur between 50 °C and 60 °C, the latter allowing gel networks to be established (Martens et al. 1982).

The denaturation of constituent muscle proteins generally brings about macroscopic changes in the structure of meat that may variously act to reduce or promote its permeability to enzymatic secretions and its ease of digestion. Transverse shrinkage of muscle fibres commences at 40 °C (Bendall and Restall 1983). Longitudinal shrinkage of perimyceal collagen with concomitant longitudinal shrinkage of muscle fibres commences around 60 °C (Mohr and Bendall 1969) causing unrestrained fasciculi to shrink to one quarter of their previous length. The latter process is coincident with the denaturation of collagen in the epimyceum and perimyceum which occurs between 53 °C and 63 °C. These heat-induced changes, along with age-related changes in collagen structure, influence the overall mechanical properties and texture of the meat (Purslow 1985). They also influence the physical state of exudates of sarcoplasmic fluid within the meat, i.e. the locality and extent of their gelation and the quantities of globular proteins that are extravasated into this fluid (Tornberg 2005).

The variation with age in the manner in which collagen denatures results from differences in the degree of cross-linking of the component tropocollagen subunits. Hence collagen fibres from young animals dissolve more readily as they have fewer few heat-stable cross-links, enabling them to more readily reorganise into the gel state while the greater number of such cross-links in the collagen of older animals inhibits gel formation (Sims and Bailey 1981). Provided sufficient water is present, denatured collagen monomers and denatured molecules of globular proteins can form symmetrical patterns of association forming watery and readily digestible gels. The structure of such gels may vary from a fragile network of fine ‘strands’ to a network of coarser aggregated strands (Clark 1998), the latter exhibiting more ‘solid-like’ behaviour.

In a number of proteins with a discrete molecular configuration, notably the globular type, denaturation can occur over a more narrow temperature or pH range; the lack of intermediate states suggesting that unfolding occurs in a concerted manner once a particular bond energy has been exceeded (Damodaran 2008). Hence denaturation of globulins tends to be more reversible and they may regain their original folded active state when cooled, even after heating to 100 °C for short periods. Longer periods of heating bring about permanent denaturation of globulins with irreversible chemical changes in amino acid residues including deamidation, bond cleavage and chemical disintegration. These conformational changes may improve the access of digestive enzymes to susceptible regions of the protein molecule, e.g. the cooking of eggs reduces the proportion of protein leaving the ileum in ileostomised subjects from 35 to 6% (Evenepoel et al. 1999).

Globular proteins generally possess a thermodynamically stable tertiary structure that shields hydrophobic side chains from the surrounding aqueous environment and sequesters them in a single non-polar region. Higher processing temperatures can sometimes create thermodynamic conditions which allow these structures to partially unfold and associate with adjacent molecules to form an alternate more complex structure that better allows the shielding of hydrophobic portions of the constituent amino acid chains. This can lead to widespread agglomeration and precipitation. Alternately, the formation of a symmetrical pattern of associations between adjacent molecules can lead to the development of a coalesced structure which binds any redundant water of solution, i.e. the formation of a gel.

Certain globular proteins, e.g. lactoferrin, may orient their polar and non-polar regions across the oil–water interfaces of fatty emulsions and hence act as emulsifying agents (see Chap. 5). Such proteins may also stabilise the droplets within the emulsion by giving them a net positive charge. However, the manner in which the protein is orientated across the interface may sterically limit proteolysis, promoting the cleavage and release of the projecting hydrophilic portion of the molecules (Sarkar et al. 2009). Hence the pattern of proteolysis may alter when other nutrients notably emulsified fats are present.

4.3.2 Maillard Reactions

Maillard reactions or non-enzymatic food browning frequently occur during cooking, and are time and temperature dependent (Ledl and Schleicher 1990). They occur when proteins are heated in the presence of reducing carbohydrates causing the free amino group of a constituent amino acid of a protein, e.g. the epsilon amino group of lysine, to condense with the carbonyl group of a reducing sugar, e.g. glucose (Öste and Sjödin 1984).

While cooking is associated with a general improvement in the digestibility of foods, the Maillard reaction may decrease the local availability of essential amino acids such as lycine and reduce the digestibility of food products (Hurrell and Carpenter 1977; Öste et al. 1987; Öste and Sjödin 1984). Hence it may hinder the solution of proteins, the ingress of digestive fluid and digestion of proteinaceous particles.

Maillard reactions can also occur in vivo (Zhang et al. 2009), though they are not so extensive. They produce advanced products of glycation (AGEs). When proteins that contain such products are eaten, they may produce carcinogenic compounds following bacterial proteolysis in the colon (Tuohy et al. 2006).

4.3.3 Antinutrients

Antinutrients are chemicals specifically synthesised by plants to deter herbivores. Antinutrient substances include toxins and hormonal agents as well as those which reduce the absorption and digestibility of particular nutrients (Liener 1994). A number of the latter directly influence the digestibility of plant proteins by forming indigestible complexes with them. These agents may also act indirectly by forming similar complexes with the digestive enzymes of the consumer.

Tannin antinutrients are produced by a number of plants specifically to sequester proteins, impair their digestibility and render them insoluble (Hagerman and Butler 1981). Hence they may combine with dietary proteins rendering them indigestible or inactivate digestive enzymes (Ahmed et al. 1991). The binding of tannins with proteins commonly occurs by stacking of the tannin polyphenol ring against the pyrrolidone ring of proline residues (Baxter et al. 1997; Charlton et al. 2002). Hence in situations where this binding occurs at surfaces of proteinaceous particles, it may influence the dissolution of proteins and the ingress of digestive fluids and enzymes. Thus the precipitation of black bean globulins with black bean tannins markedly reduces their susceptibility to digestion by pepsins although they could be digested by a mixture of small intestinal enzymes trypsin, chymotrypsin and peptidases (Aw and Swanson 1985).

The status of phytates as antinutrients is less secure. They are oligosaccharide products of enzymatic digestion and hence may be simple by products of the digestion of structural compounds. However, they are potent chelating agents and are known to sequester iron and other essential dietary minerals. They may also decrease the solubility (Bourdillon 1951) and digestibility (Kratzer 1965) of a number of proteins, e.g. casein and bovine serum albumin (Knuckles et al. 1985).

4.4 The Digestion of Proteins

Dietary proteins undergo enzymatic cleavage in the lumen of the stomach and the small intestine. The oligopeptides produced by these enzymes must traverse the unstirred water layer and mucus layer before undergoing subsequent cleavage to constituent amino acids by enzymes associated with the enterocyte brush border of the small intestine (Stipanuk 2006). Although the bulk of oligosaccharides are reduced to constituent amino acids, it is recognised that a number of oligopeptides resist enzymatic breakdown to their constituent amino acids and may be absorbed directly (Meisel 1986; Zioudrou et al. 1979).

Given that the conformation of proteins is influenced by pH, it is noteworthy that proteolysis in the lumen occurs firstly in an acidic and subsequently in an alkaline aqueous phase. Such a succession may constitute a digestive strategy designed to maximise proteolytic capability, the two phases acting sequentially to secure maximal breakdown of resistant proteins, a hypothesis that is supported by studies on the digestion of collagen (see below).

4.4.1 Gastric and Small Intestinal Digestion

4.4.1.1 Meat Proteins

The acidic conditions within the stomach created by the secretion of hydrochloric acid are sufficient to dissolve and induce the denaturation of fibrous proteins such as myosin and actin. Hence peptide regions that normally are shielded within their α-helical tertiary structures become accessible to proteases (Bender 1978; Gitler 1964). The triple helical structure of collagen is more resistant to solution and denaturation under these conditions.

A number of workers have reported that native collagens are not digested by animal pepsin or pancreatin (Asghar and Henrickson 1982). Were this so, the digestion of meat would be impeded by the disposition of collagens within and around uncooked muscle. However, other workers have reported that collagens are successfully digested in the stomach (Harkness et al. 1978). The discrepancy results from the limitations in the action of gastric proteases insofar as their ability to degrade native collagens. Hence subsequent investigation showed that collagens must be in solution before they can undergo denaturation or digestion (Etherington 1984). Gastric pepsin is unable to cleave peptide bonds in the helical region of native collagens but can solubilise them by cleavage in their non-helical regions (Weiss 1976). However, such cleavage can only occur when the lumen pH is below 2.5 and after they have undergone hydrolysis and hydration by HCl (Etherington 1984; Etherington and Bailey 1982). It has been suggested that a gastric luminal pH below 4.5 is sufficient to allow hydrolysis of labile aldimine cross-links in collagen, allowing the molecular architecture to swell and the non-helical regions to undergo limited cleavage by pepsin with subsequent solution and denaturation of the resultant monomers (Etherington 1977). As noted earlier, the latter process may be incomplete in the stomach, the remaining material being broken down on subsequent exposure to trypsin from pancreatin in the small intestine. Similar dynamics may account for the slow degradation of elastin by pepsin (Abderhalden and Strauch 1911).

The importance of gastric acid hydrolysis is underscored in experiments where the acid secretion in the stomach of rats is inhibited by omeprazole. Thus the digestibility of finely powdered insoluble collagen in vivo is reduced (71%) compared with that in normal rats (95%) (Reutersward and Fabiansson 1985). It is noteworthy that the digestibility of soluble egg protein and gelatin did not differ between the normal and treated rats indicating that acid hydrolysis was not necessary for the efficient digestion of these proteins. Hence while the acidity of the stomach lumen has little influence on more readily digestible proteins, it is important in the digestion of more resistant proteins. It is noteworthy that this effect may be limited in situations where larger pieces of meat are consumed and diffusion paths for secreted acids are correspondingly extended.

The important contribution of intragastric denaturation to the efficient digestion of native collagen is further underscored by work showing that exposure of small pieces of meat to the action of pancreatic juice in vitro brings about digestion of the sarcolemma but leaves the surrounding collagenic connective tissue intact (Baumstark and Cohnheim 1910). Hence the gastric proteolysis of raw meat may be slowed by accumulation of layers of undigested collagen and elastin that are resistant to proteolysis. This may occur particularly during the early postprandial period before the reduction of the luminal gastric pH to below 3 and if it is uncooked or incompletely denatured during prior processing.

The digestion of cooked meat may not be subject to these limitations as a result of extensive denaturation of any contained collage (see the previous section). Moreover, postmortem changes in the structure of meat may promote its digestion. Hence postmortem rigour by inducing transverse shrinkage of muscle fibres creates gaps between the sarcolemma and the surrounding endomysium (Offer and Cousins 1992; Offer and Knight 1988). Again the postmortem operation of tissue collagenases may reduce the collagen content (Laakkonen et al. 1970) of the endomysium and aid permeation of gastric proteases.

4.4.1.2 Plant Proteins

Plant proteins, particularly those in legume grains, generally have lower in situ digestibilities than those in animal tissue (Friedman 1996). This may result from their relative insolubility, their intracellular organisation and the low permeability of plant cell walls and the seed coat. Hence vegetable matter generally requires cooking or some other form of processing to increase the digestibility of the contained protein. Boiling and subsequently the freezing of legume seeds increases their digestibility to more than 95% (Khattab et al. 2009).

The separation of the protein from the plant material in which it is normally contained markedly increases its digestibility. The digestibility of purified native plant proteins could be as high as 95% and similar to that of bovine serum albumin (98%) (Carbonaro et al. 2005; Carbonaro et al. 2000). Moreover, studies have shown that the digestibilities of processed animal and plant proteins do not differ significantly in rats or in humans (Bodwell et al. 1980). However, the somewhat lower values for digestibility of plant proteins and the fact that the in vivo digestibility of purified 7S and 11S proteins from two bean species (Carbonaro et al. 2005; Carbonaro et al. 2000) were reduced by 3 to 13% when they were denatured suggest that the structural properties of plant proteins may have some influence on digestibility.

As noted earlier, plant proteins generally occur in discrete bodies that are distributed in a cellular matrix composed of other plant materials. Hence their digestibility depends upon the size and mechanical characteristics of the surrounding heterogeneous matrix, notably the ease with which it may be permeated, softened and eroded. The rate at which mass is lost from large pieces of mechanically resistant protein-containing material such as dry roasted almonds is low and depends upon the level of shear that is applied, i.e. on the manner in which digesta is physically processed (Kong and Singh 2009). Moreover, the rate at which less mechanically durable matrices are eroded is more readily influenced by shear and processing (Kong and Singh 2009).

4.4.1.3 Dairy Proteins

The rates at which the constituent proteins of cow's milk are digested differ in human subjects. Thus, for example, the rate at which amino acids were absorbed from a meal containing casein proteins was markedly slower than that from a meal containing whey proteins (Boirie et al. 1997). This may result from their differing behaviour in the adult human gastric environment. Theoretical considerations indicate that casein micelles will aggregate in the stomach. Moreover, the samples of gastric digesta obtained following the consumption of casein contain fragmented particles of casein (Calbet and Holst 2004). There is disagreement as to whether such aggregation influences gastric emptying, some researchers reporting that casein delays gastric emptying (Mahé et al. 1996), others reporting that casein empties in a similar exponential fashion and at a similar rate to whey (Calbet and Holst 2004). The latter workers suggested that the fine casein curd particles are emptied with the liquid fraction from the stomach. At all events it seems that casein aggregates form particles in the gastric environment. Hence some of the delay in absorption may result from the heterogeneous structure of the casein particle restricting the access of gastric protease. A further possibility is that some oligopeptides produced from the gastric proteolysis of casein influence gastrointestinal motility (Daniel et al. 1990).

The rate of digestion of constituent proteins in ultra-high temperature (UHT)-treated milk has been found to be higher than that of pasteurised or microfiltered milk (Lacroix et al. 2008). UHT causes whey proteins to denature and interact with casein micelles to modify their aggregation behaviour and the mechanical properties of the aggregate (Guyomarc'h et al. 2003). It was suggested that the greater rate of proteolysis of UHT-treated milk resulted from a large increase in the number of very small, soluble particles that were formed from small weak aggregates (Lacroix et al. 2008). However, it should also be noted that isolated β-lactoglobulin (a major whey protein in the milk of ungulates) also becomes less resistant to enzymatic proteolysis after heating to 80–90 °C and that this is known to result from changes in its molecular conformation (Reddy et al. 1988).

4.4.2 Colonic Digestion

The majority of proteinaceous material entering the large intestine originates from the diet and the amount is dependent on both the quantity (Gibson et al. 1976) and form of ingested protein (Chacko and Cummings 1988). Other proteins are of endogenous origin and include enzymes, mucins and exfoliated cells. On entering the colon, proteinaceous material undergoes digestion by bacterial and residual pancreatic proteases, the resulting short peptides and amino acids being the substrates for further bacterial fermentative digestion (Macfarlane et al. 1988). The end-products of such fermentative digestion are short chain fatty acids (SCFA), ammonia and a range of potentially carcinogenic metabolic compounds including phenolics, amines and sulphides (Hughes et al. 2000). A proportion of the SCFAs produced as a result of amino acid fermentation are branched chain including isobutyrate, 2-methylbutyrate and isovalerate (Macfarlane et al. 1992). While the fermentative digestion of carbohydrates appears to occur predominantly in the proximal colon, the formation of such SCFAs from amino acids becomes more pronounced as carbohydrate becomes depleted in the distal colon (Macfarlane et al. 1992).

The rate of excretion of nitrogen in the stool has been reported to be 1–7 g/day (Pinter and McLean 1968), the major proportion of which comprises bacterial protein. The proportion of non-bacterial nitrogen in the stool increases with the consumption of red meat (Hughes et al. 2000). Bacterial protein is unavailable for further digestion and absorption within the colon. Hence it is voided by humans but it is selectively retained in the proximal colon or caecum in certain herbivores to be voided in bulk and subsequently re-ingested, i.e. coprophaged (Stevens and Hume 1995).

References

Abderhalden E, Strauch FW (1911) Weitere studien über die wirkung der fermente des magensaftes. II. Mitteilung. Hoppe-Seyler´s Zeitschrift für physiologische Chemie 71:315-338

Ahmed AE, Smithard R, Ellis M (1991) Activities of enzymes of the pancreas, and the lumen and mucosa of the small intestine in growing broiler cockerels fed on tannin-containing diets. Br J Nutr 65:189-197

Asghar A, Henrickson RL (1982) Chemical, biochemical, functional, and nutritional characteristics of collagen in food systems. Adv Food Res 28:231-372

Aw TL, Swanson BG (1985) Influence of tannin on *Phaseolus vulgaris* protein digestibility and quality. J Food Sci 50:67-71

Bailey AJ, Restall DJ, Sims TJ, Duance VC (1979) Meat tenderness: Immunofluorescent localisation of the isomorphic forms of collagen in bovine muscles of varying texture. J Sci Food Agric 30:203-210

Baumstark R, Cohnheim O (1910) Über bindegewebsverdauung. Hoppe-Seyler´s Zeitschrift für physiologische Chemie 65:477-482

Baxter NJ, Lilley TH, Haslam E, Williamson MP (1997) Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. Biochemistry 36:5566-5577

Bendall JR, Restall DJ (1983) The cooking of single myofibres, small myofibre bundles and muscle strips from beef *M. Psoas* and *M. Sternomandibularis* muscles at varying heating rates and temperatures. Meat Science 8:93-117

Bender AE (1978) Food processing and nutrition. Academic Press, London

Bodwell C, Satterlee L, Hackler L (1980) Protein digestibility of the same protein preparations by human and rat assays and by in vitro enzymic digestion methods. Am J Clin Nutr 33:677-686

Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrère B (1997) Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci USA 94:14930-14935

Bourdillon J (1951) A crystalline bean seed protein in combination with phytic acid. J Biol Chem 189:65-72

Calbet JAL, Holst JJ (2004) Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. Eur J Nutr 43:127-139

Carbonaro M, Grant G, Cappelloni M (2005) Heat-induced denaturation impairs digestibility of legume (*Phaseolus vulgaris* L and *Vicia faba* L) 7S and 11S globulins in the small intestine of rat. J Sci Food Agric 85:65-72

Carbonaro M, Grant G, Cappelloni M, Pusztai A (2000) Perspectives into factors limiting in vivo digestion of legume proteins: Antinutritional compounds or storage proteins? J Agric Food Chem 48:742-749

Chacko A, Cummings JH (1988) Nitrogen losses from the human small bowel: Obligatory losses and the effect of physical form of food. Gut 29:809-815

Charlton AJ, Baxter NJ, Khan ML, Moir AJG, Haslam E, Davies AP, Williamson MP (2002) Polyphenol/peptide binding and precipitation. J Agric Food Chem 50:1593-1601

Clark AH (1998) Gelation of globular proteins. In: Hill SE, Ledward DA, Mitchell JR (eds) Functional properties of food macromolecules. Aspen, Gaithersburg, pp 77-142

Dalgleish DG, Spagnuolo PA, Douglas Goff H (2004) A possible structure of the casein micelle based on high-resolution field-emission scanning electron microscopy. Int Dairy J 14:1025-1031

Damodaran S (2008) Amino acids, peptides and proteins. In: Damodaran S, Parkin KL, Fennema OR (eds) Fennema's food chemistry. CRC Press, Boca Raton

Daniel H, Vohwinkel M, Rehner G (1990) Effect of casein and β-casomorphins on gastrointestinal motility in rats. J Nutr 120:252-257

El Jabri M, Abouelkaram S, Damez JL, Berge P (2010) Image analysis study of the perimysial connective network, and its relationship with tenderness and composition of bovine meat. J Food Eng 96:316-322

Etherington DJ (1977) The dissolution of insoluble bovine collagens by cathepsin BI, collagenolytic cathepsin and pepsin: The influence of collagen type, age and chemical purity on susceptibility. Connect Tissue Res 5:135-145

Etherington DJ (1984) The contribution of proteolytic enzymes to postmortem changes in muscle. J Anim Sci 59:1644-1650

Etherington DJ, Bailey AJ (1982) Metabolism of rat skin collagen: The effect of arrested development and subsequent catch-up growth on the stability of the connective tissue fibers. Coll Relat Res 2:507-522

Evenepoel P, Claus D, Geypens B, Hiele M, Geboes K, Rutgeerts P, Ghoos Y (1999) Amount and fate of egg protein escaping assimilation in the small intestine of humans. Am J Physiol 277:G935-943

Fox PF (2003) Milk proteins: General and historical aspects. In: Fox PF, P.L.H. M (eds) Advanced dairy chemistry: Proteins Part A. Kluwer, New York, pp 1-48

Friedman M (1996) Nutritional value of proteins from different food sources. A review. J Agric Food Chem 44:6-29

Gibson JA, Sladen GE, Dawson AM (1976) Protein absorption and ammonia production: The effects of dietary protein and removal of the colon. Br J Nutr 35:61-65

Gitler C (1964) Protein digestion and absorption in nonruminants. In: Munro HN, Allison JB (eds) Mammalian protein metabolism. Academic Press, New York, pp 35-69

Guyomarc'h F, Law AJR, Dalgleish DG (2003) Formation of soluble and micelle-bound protein aggregates in heated milk. J Agric Food Chem 51:4652-4660

Hagerman AE, Butler LG (1981) The specificity of proanthocyanidin-protein interactions. J Biol Chem 256:4494-4497

Harkness ML, Harkness RD, Venn MF (1978) Digestion of native collagen in the gut. Br Med J 19:240-243

Hughes R, Magee EAM, Bingham S (2000) Protein degradation in the large intestine: Relevance to colorectal cancer. Curr Issues Intest Microbiol 1:51-58

Hurrell RF, Carpenter KJ (1977) Nutritional significance of cross-link formation during food processing. Adv Exp Med Biol 86:225-238

Kauzmann W (1959) Some factors in the interpretation of protein denaturation. In: Anfinsen CB (ed) Adv protein chem. Academic press, New York, pp 1-64

Kong F, Singh RP (2009) Digestion of Raw and Roasted Almonds in Simulated Gastric Environment. Food Biophysics 4:365-377

Khattab RY, Arntfield SD, Nyachoti CM (2009) Nutritional quality of legume seeds as affected by some physical treatments, part 1: Protein quality evaluation. LWT-Food Sci Technol 42:1107-1112

Knuckles BE, Kuzmicky DD, Betschart AA (1985) Effect of phytate and partially hydrolyzed phytate on *in vitro* protein digestibility. J Food Sci 50:1080-1082

Kratzer FH (1965) Soybean protein-mineral interrelationships. Fed Proc 24:1498-1500

Laakkonen E, Sherbon JW, Wellington GH (1970) Low-temperature, long-time heating of bovine muscle. 3. Collagenolytic activity. J Food Sci 35:181-184

Lacroix M, Bon C, Bos C, Leonil J, Benamouzig R, Luengo C, Fauquant J, Tome D, Gaudichon C (2008) Ultra high temperature treatment, but not pasteurization, affects the postprandial kinetics of milk proteins in humans. J Nutr 138:2342-2347

Ledl F, Schleicher E (1990) New aspects of the Maillard reaction in foods and in the human body. Angew Chem Int Ed Engl 29:565-594

Liener IE (1994) Implications of antinutritional components in soybean foods. Crit Rev Food Sci Nutr 34:31-67

Macfarlane GT, Allison C, Gibson SAW, Cummings JH (1988) Contribution of the microflora to proteolysis in the human large intestine. J Appl Microbiol 64:37-46

Macfarlane GT, Gibson GR, Beatty E, Cummings JH (1992) Estimation of short-chain fatty acid production from protein by human intestinal bacteria based on branched-chain fatty acid measurements. FEMS Microbiol Lett 101:81-88

Mahé S, Roos N, Benamouzig R, Davin L, Luengo C, Gagnon L, Gausserges N, Rautureau J, Tome D (1996) Gastrojejunal kinetics and the digestion of [15N]β-lactoglobulin and casein in humans: The influence of the nature and quantity of the protein. Am J Clin Nutr 63:546-552

Marcone MF (1999) Biochemical and biophysical properties of plant storage proteins: A current understanding with emphasis on 11S seed globulins. Food Res Int 32:79-92

Martens H, Stabursvik E, Martens M (1982) Texture and colour changes in meat during cooking related to thermal denaturation of muscle proteins. J Text Stud 13:291-309

Meisel H (1986) Chemical characterization and opioid activity of an exorphin isolated from in vivo digests of casein. FEBS Lett 196:223-227

Mohr V, Bendall JR (1969) Constitution and physical chemical properties of intramuscular connective tissue. Nature 223:404-405

Moughan PJ, Cranwell PD, Smith WC (1991) An evaluation with piglets of bovine milk, hydrolyzed bovine milk, and isolated soybean proteins included in infant milk formulas. II. Stomach-emptying rate and the postprandial change in gastric pH and milk-clotting enzyme activity. J Pediatr Gastroenterol Nutr 12:253-259

Offer G, Cousins T (1992) The mechanism of drip production: Formation of two compartments of extracellular space in muscle post mortem. J Sci Food Agric 58:107-116

Offer G, Knight P (1988) The structural basis of water-holding in meat. In: Lawrie RA (ed) Developments in meat science - 4. Elsevier, New York, pp 63–171

Okita TW, Rogers JC (1996) Compartmentation of proteins in the endomembrane system of plant cells. Annu Rev Plant Physiol Plant Mol Biol 47:327-350

Öste RE, Miller R, Sjöström H, Noren O (1987) Effect of Maillard reaction products on protein digestion. Studies on pure compounds. J Agric Food Chem 35:938-942

Öste RE, Sjödin P (1984) Effect of Maillard reaction products on protein digestion. *In vivo* studies on rats. J Nutr 114:2228-2234

Pinter KG, McLean A (1968) Correlation between weight, nitrogen, and fat content of stool. Am J Clin Nutr 21:1310-1313

Purslow PP (1985) The physical basis of meat texture: Observations on the fracture behaviour of cooked bovine *M. Semitendinosus.* Meat Science 12:39-60

Purslow PP (2002) The structure and functional significance of variations in the connective tissue within muscle. Comp Biochem Physiol A 133:947-966

Reddy IM, Kella NKD, Kinsella JE (1988) Structural and conformational basis of the resistance of β-lactoglobulin to peptic and chymotryptic digestion. J Agric Food Chem 36:737-741

Reutersward AL, Fabiansson S (1985) *In vivo* digestibility of insoluble collagen from bovine tendon as influenced by the inhibition of gastric acid secretion. J Food Sci 50:1523-1525

Sarkar A, Goh KKT, Singh RP, Singh H (2009) Behaviour of an oil-in-water emulsion stabilized by [beta]-lactoglobulin in an *in vitro* gastric model. Food Hydrocolloids 23:1563-1569

Shewry P, Napier J, Tatham A (1995) Seed storage proteins: Structures and biosynthesis. Plant Cell 7:945-956

Shewry PR (2003) Tuber storage proteins. Ann Bot 91:755-769

Sims TJ, Bailey AJ (1981) Connective tissue. In: Lawrie RA (ed) Developments in meat science - 2. Applied Science Publishers, London, pp 29-59

Stevens CE, Hume ID (1995) Comparative physiology of the vertebrate digestive system. Cambridge University Press, New York

Stipanuk MH (2006) Biochemical, physiological, & molecular aspects of human nutrition. Saunders-Elsevier, St. Louis

Strasburg G, Xiong YL, Chiang W (2008) Physiology and chemistry of edible muscle tissues. In: Damodaran S, Parkin KL, Fennema OR (eds) Fennema's food chemistry. CRC Press, Boca Raton, pp 923-973

Tornberg E (2005) Effects of heat on meat proteins - implications on structure and quality of meat products. Meat Science 70:493-508

Tuohy KM, Hinton DJ, Davies SJ, Crabbe MJC, Gibson GR, Ames JM (2006) Metabolism of Maillard reaction products by the human gut microbiota - implications for health. Mol Nutr Food Res 50:847-857

Walstra P (1979) The voluminosity of bovine casein micelles and some of its implications. J Dairy Res 46:317-323

Weiss JB (1976) Enzymic degradation of collagen. Int Rev Connect Tissue Res 7:101-157

Zhang Q, Ames JM, Smith RD, Baynes JW, Metz TO (2009) A perspective on the Maillard reaction and the analysis of protein glycation by mass spectrometry: Probing the pathogenesis of chronic disease. J Proteome Res 8:754-769

Zioudrou C, Streaty R, Klee W (1979) Opioid peptides derived from food proteins. The exorphins. J Biol Chem 254:2446-2449

Chapter 5
Colloidal Dynamics and Lipid Digestive Efficiency

Contents

5.1 Introduction

Dietary lipids in both unemulsified and emulsified form are an integral part of our daily food intake. The term lipid is itself a generic label to describe a group of naturally occurring, predominantly apolar molecules, which contribute to a number of biological functions. The common molecular element of dietary lipids is the aliphatic hydrocarbon chain, which can vary in chain length and degree of saturation.

With Contribution by Matt Golding and Tinothy Wooster

R. G. Lentle, P. W. M. Janssen, *The Physical Processes of Digestion,*
DOI 10.1007/978-1-4419-9449-3_5, © Springer Science+Business Media, LLC 2011

Variation in chain length is a determining factor in the functional properties of lipids, including melting point, relative aqueous solubility, and packing arrangement in the crystalline state. For most naturally occurring lipids, chain length varies between 4 and 28 carbon atoms. Also of consequence is the molecular arrangement at the terminus of the hydrocarbon chain. Free fatty acids (FFAs) represent the simplest lipid components, comprising a carboxylic acid group at the 1 carbon position, which imparts a polar component to the molecule. This is most pronounced in short-chain fatty acids, which are soluble in water. However, with increasing chain length the molecule becomes increasingly hydrophobic and solubility decreases accordingly. From a dietary perspective, FFAs are only a relatively small component of our intake of lipids, with triglycerides being the main constituent.

Triglycerides are produced through the esterification of three molecules of fatty acid with a molecule of glycerol. Triglyceride molecules are considerably less polar than fatty acids and are therefore insoluble in water. The macromolecular arrangement of triglycerides is very much dependent on the three fatty acids that comprise each triglyceride molecule, whether identical or mixed, and the relative differences in chain length and degree of saturation. Triglyceride fractions containing high proportions of saturated or long-chain fatty acids are most likely to be crystalline under ambient conditions and are commonly termed fats, while triglycerides based on shorter-chain or unsaturated fatty acids are commonly liquid at ambient conditions and are termed oils. Typical dietary fats and oils also contain low concentrations of other lipid components, including apolar lipids, such as sterols and non-digestible waxes, as well as polar lipids, such as monoglycerides and phospholipids. A particular structural aspect of polar lipids is their amphipathic nature. This enables them to assemble into mesophase and colloidal structures in solution, which has important consequences for digestion and metabolism of lipids.

As with other macronutrients, triglycerides must be converted to a physical and molecular form that allows them to readily transit the unstirred water layer and epithelium, and be absorbed. Triglycerides, being poorly water soluble, are not able to directly transit these layers. Instead, hydrolysis of lipids is achieved through the action of gastric and intestinal lipases, which act on the surfaces of emulsified lipids to liberate the component fatty acids and monoglycerides. The subsequent assembly of these more polar lipid fractions into discrete nano-structured mixed micelles enables their transit across the intestinal lumen.

The hydrolysis of triglycerides results primarily from the action of gastric and pancreatic lipases, with salivary lipases, esterases and phosphatases playing a lesser role. Lipolysis by acid-stable gastric lipase accounts for between 10–30% of total fat hydrolysis. Subsequent digestion occurs within the small intestine by the continued action of gastric lipase and by co-lipase-dependant pancreatic lipase. The efficiency of this process is such that only small amounts of undigested fat enter the colon (Armand 2007; Carey et al. 1983; Mu and Høy 2004). The proportion of dietary fat absorbed is typically greater than 95% in healthy adults, but is lower in neonates (between 65 and 80%) as a consequence of incomplete development of liver function in the newborn.

Digestive lipases are only active at oil–water interfaces. Hence lipolytic activity is relatively inefficient with bulk oils owing to the restricted area of the oil–water interface. Increasing the surface area of this interface by emulsification will improve the efficiency of lipolysis. While a number of foods contain emulsified fats, notably milk, a significant proportion contain unemulsified fats, e.g. meat fat and vegetable oils, and require a degree of emulsification to be efficiently absorbed (Golding and Wooster 2010; McClements et al. 2009; Norton et al. 2007; Singh et al. 2009).

The ease with which liquid fat is emulsified within the digestive tract is influenced by the magnitude of forces applied during digestive processing; by the temperature in the gut; and by the presence of amphiphilic molecules. Amphiphiles that are relevant to digestive emulsification include those of endogenous origin, e.g. salivary proteins, FFAs, monoglycerides, bile salts and phospholipids; and those of exogenous origin, namely dietary proteins, other non-protein biopolymers and lipid-derived surfactants (Mun et al. 2006). Although lipases readily associate with bile at the oil–water interface when co-lipase is present, the presence of exogenous amphiphilic agents renders the overall physical processing of fats more complex. While these agents may promote emulsification, they may also inhibit the interfacial adsorption of lipases, which is necessary for lipolysis. Further complexity results from the fact that the behaviour of such amphiphilic agents, and hence their ability to orient across the oil–water interface, is influenced by the biochemical conditions within the digesta, notably the pH and the action of digestive enzymes.

The nature of fat within the droplet may also influence the efficiency of lipolysis. Triacylglycerols adopt stereochemical conformations at oil–water interfaces (Fahey and Small 1986) that differ from the ‘tuning fork’ conformation, which occurs within the bulk lipid phase of the drop (Kodali et al. 1989). Hence in droplets formed from triacylglycerols that possess one short fatty acid chain, e.g. in butterfat, the short chain orients into the aqueous phase and may be more rapidly digested. Nuclear magnetic resonance (NMR) studies indicate that for triolein the sn1 and sn3 ester groups project slighter more into the aqueous layer than does the sn2 ester group, which may account for the site specificity of the hydrolytic action of the gut lipases (Hamilton et al. 1983; Hamilton and Small 1981). Interestingly, the presence of a medium-chain fatty acid in a triglyceride projecting into the hydrophobic portion of the drop alongside of longer-chain fatty acids causes a local reduction in melting point and an increase in surface fluidity of the droplet (Fahey and Small 1988). This raises the question as to whether the sequence of fatty acids in triacylglycerol can further influence the efficiency of lipolysis.

While some products of lipolysis are water soluble, others, notably long-chain fatty acids, are relatively insoluble, and hence cannot diffuse into the fluid phase and across the lumen. Although emulsification serves to improve the efficiency of lipid hydrolysis, the emulsion droplets cannot traverse the unstirred water layer or the apical membrane of the enterocytes. However, the polar nature of the products of triglyceride lipolysis allows them to associate with endogenous biosurfactants at the surface of emulsion droplets, and to be released as small thermodynamically stable assemblies (so-called ‘mixed micelles’). These assemblies, being of the order of nanometres in diameter, are more likely to transit the unstirred water layer and interact with the apex of the enterocyte. The dynamics by which these molecules efficiently traverse the intestinal lumen and the unstirred water layer are incompletely understood. It is known that the dynamic nature of mixed micelles serves to raise ambient levels of lipolytic products at the surface of the enterocyte (Carey et al. 1983). However, we have only limited understanding of the means by which the products of lipolysis detach from the emulsion interface and self-assemble into bile micelles and vesicles (Madenci and Egelhaaf 2010); how these structures accumulate polar lipid moieties, such as cholesterol; and how they interact with or penetrate the unstirred water layer. Recent work suggests that pH may be a principal driver, the size and form of structures changing from liquid crystalline form with a hydrodynamic radius ~120 nm to vesicles of radius ~60 nm at pH around 7–8 (Salentinig et al. 2010).

This chapter will review the sequence of physical processes within the successive segments of the gastrointestinal tract that contribute to the development of a colloidal state and the enzymatic degradation of ingested fats. We will also discuss the biophysical processes that govern the assembly, transit and absorption of lipolytic products.

5.2 The Oral Processing of Fats and Oils

5.2.1 The Oral Environment

Digestion of ingested lipids commences in the mouth where foods and liquids are exposed to physical forces, enzymatic and mucosal secretions, and undergo warming or cooling to ~37 °C (Hutchings and Lillford 1988). Oral processing also provides an opportunity for sensory evaluation of texture and taste. The oral cavity is remarkably sensitive to changes in the physical properties of foods, notably the emulsification status and physical form of fats and oils (Malone et al. 2003). Such sensitivity is likely to be evolutionarily relevant, given the importance of this macronutrient as a source of energy and the need to deliver lipids into the stomach in some form of emulsion to ensure that intragastric lipolysis can promptly commence.

5.2.2 The Oral Processing of Non-Emulsified Oils

While bulk free oil is not generally consumed as part of a regular diet, examining the manner in which the physical form of free oil changes during oral processing provides some insight into the changes that occur to the non-emulsified oil content of many foods.

While there are relatively few studies on the oral processing of unemulsified lipids, some research has been carried out examining changes in the physical form of oils of differing viscosity (corn and castor oil) during oral processing using video capture endoscopy (Adams et al. 2007). A rigid endoscope, connected to a fluorescence microscope, was inserted into the mouth during and after rinsing of a 5-ml aliquot of oil around the mouth for 30 s. Endoscopic images, taken after expectoration of the bulk of the oil, show that coarse oil-in-water emulsions are formed in the

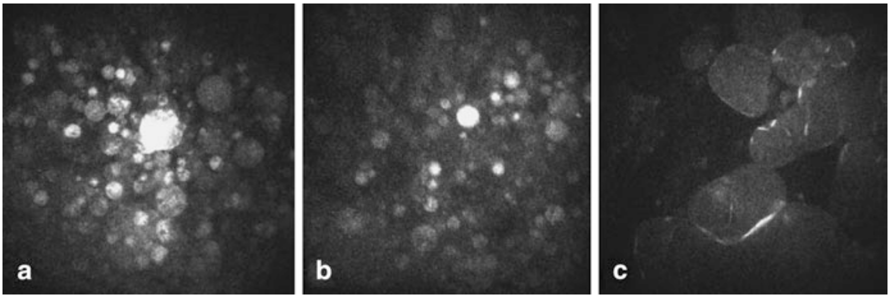


Fig. 5.1 Single frames from the video recorded from the surface of the tongue immediately after 30 s oral processing of a bulk oil sample. **a** Castor oil at the back right of the tongue, **b** corn oil at the back right of the tongue, and **c** castor oil at the tip of the tongue. Image width is 1.5 mm. (Adams et al. 2007)

remaining intraoral fluid, those from the lower viscosity oil having slightly smaller droplets (Fig. 5.1).

In situations where the volume of oil ingested is in excess of that of saliva (typically >10 ml), a coarse water-in-oil emulsion is formed. This persists even after 10 min of 'rinsing around the mouth' before inverting to form an oil-in-water emulsion containing oil droplets sized between 10 and 40 µm. This inversion may result either from dilution with saliva or the gradual involuntarily swallowing of oil during the rinsing process. Correspondingly, the initial formation of a water-in-oil emulsion results either from the low initial volume of the aqueous phase or from some limitation in the emulsifying action of saliva, e.g. the action of salivary proteins and enzymes, such as lingual lipase.

When a quantity of water is ingested along with the sample of oil, the oil-in-water emulsion is formed at the commencement of the rinsing manoeuvre (de Bruijne and Bot 1999; de Bruijne et al. 1993). Free oil is emulsified to 20–30-µm droplets after only 30 s of rinsing, the final size being similar to that found in the authors' study. Hence it seems likely that the earlier establishment of widespread elongational flow, i.e. movement of oil through occlusive gaps between upper and lower dentaries, and between tongue and palate, is the primary contributor to the oral emulsification of free oil. The shear force being applied to the droplets during the rinsing manoeuvre is estimated to be as high as 50 Pa (de Bruijne and Bot 1999; de Bruijne et al. 1993). This fits in with other estimates by these workers showing that droplets in course emulsion systems with a yield stress greater than 50 Pa could not be broken up in the mouth.

As well as applying shear, the mouth is able to promote biochemical emulsification. Several studies have shown that a number of salivary protein fractions exhibit surface activity, and hence are capable of forming interfacial films with a high degree of elasticity (Glantz 1997; Rossetti et al. 2008). However, the specific salivary proteins that produce this effect have yet to be identified.

Apart from the action of endogenous emulsifying agents, amphiphilic ingredients in a number of foods, such as surface active biopolymers or emulsifiers, can also augment the formation of oil-in-water emulsions. Similar emulsifying mechanisms may operate when foods that contain protein and free oil, such as butter and chocolate, have been chewed or sufficiently melted to cause this oil to separate from the food matrix. Such a mechanism has been demonstrated for margarine, a water-in-oil emulsion that typically contains 80% fat. The water droplets and their contents are stabilised or kinetically trapped by the crystallisation of the fat network. During oral processing at 37 °C, the fat network melts allowing the water phase to mix with saliva. Shear forces within the mouth initially promote a rapid coalescence of the dispersed aqueous phase. Once sufficient numbers of droplets have coalesced, the contained milk proteins are able to facilitate the inversion of the emulsion to an oil-in-water state (Bakker and Mela 1996; Norton et al. 2006).

5.2.3 The Perception and Oral Processing of Emulsified Foods

It seems likely that the mouth has evolved to detect the various physical forms of ingested fats, presumably to associate pleasant feelings with the consumption of an energy-rich food and reinforce its consumption. Hence changes in the macrostructure of emulsions during oral processing have been shown to elicit an array of organoleptic sensations based on texture. The lubricating effects of fine emulsions are thought to elicit sensations of creaminess and smoothness. Characteristics such as fat content, structure and lipid composition can influence the relative intensity of these sensations (Bellamy et al. 2009; Mela et al. 1994). The stability of emulsion systems is also known to influence oral sensation (van Aken et al. 2007). For example, emulsions that are stabilised by small amounts of surface active proteins, but which become unstable during oral processing, impart sensations of intense creaminess, fattiness and oiliness (Dresselhuis et al. 2008a, c). Indeed, controlled destabilisation of fats is often used to enhance the ‘fatty’ sensations elicited by formulated foods, e.g. whipped cream and cheese.

It is not known whether changes in the macrostructure of emulsions during oral processing influence subsequent digestive efficiency. Indeed, it is considered by most that changes in the structure and stability of emulsified foods within the stomach and small intestine are more likely to impact on lipolytic efficiency. However, oral processing is known to influence the physical structuring of ingested fats and influence the state in they are delivered to the stomach.

The shear forces generated during oral processing may be sufficient to cause oil droplets with poor orthokinetic stability to coalesce. Confocal imaging studies of poorly stabilised emulsions show that oil droplets coalescence during tongue–palate compression in an ex vivo pigs mouth. Moreover, the coalesced droplets tend to become trapped between the papillae of the tongue (Dresselhuis et al. 2008b) providing a fixed site for collision and coalescence with mobile droplets, further destabilising the emulsion. However, it is noteworthy that complete separation of the emulsion did not occur in this experiment and the rate of coalescence appeared to be matched by the rate of droplet break-up under oral shear, the droplet size being maintained at around 20–40 μm.

While oral processing does not influence the size of individual droplets in more stable emulsions, it can induce other structural changes that are dependent upon the nature of the oil–water interface (Sarkar et al. 2009a; Silletti et al. 2007; Vingerhoeds et al. 2005). For instance, while emulsions with highly negatively charged interfacial layers do not change during oral processing, stable emulsions with smaller negative or neutral surface charges are more prone to flocculation as a result of suppression of electrostatic repulsion. These emulsions may also undergo a form of reversible depletion flocculation under static conditions. The driving force for this depletion is thought to be the exclusion of high molecular weight salivary mucins from between droplets in a manner that is analogous to other forms of polymer depletion (Dickinson 1996). In such cases, exclusion of non-adsorbed polymer from the intervening space between emulsion droplets leads to an osmotic pressure gradient between the depleted region and the surrounding solvent. This results in a weakly attractive force between droplets, which can lead to aggregation. However, as depletion interactions are weak and reversible, they are usually disrupted under orthokinetic conditions, and it is likely that emulsion flocculation of this type would not occur under normal conditions of oral processing. Similarly, it is not known whether depletion effects arising from the exclusion of mucosal biopolymers and promoting droplet association are present within the stomach, or whether gastric biomechanics are sufficient to disrupt emulsion flocculation arising from this mechanism.

Positively charged emulsions are also susceptible to aggregation, in this case, from an interaction between negatively charged salivary proteins and the positively charged interface of the emulsion droplets (Silletti et al. 2007). This type of flocculation has been shown to influence viscosity and mouthfeel (Vingerhoeds et al. 2009). While it may be possible to manipulate texture through the control of electrostatic interfacial interactions, it should be noted that very few edible emulsifiers carry a positive charge at neutral pH.

5.3 The Gastric Processing of Fats and Oils

5.3.1 Gastric Biochemistry and Relationship to Lipid Structure

Gastric lipase is stable over a pH range of 3–7, with optimal activity around 5.4 (Carriere et al. 1993). Hence gastric lipolysis is most efficient immediately after eating, when the pH of the proximal stomach lumen is high, and may continue in the more alkaline conditions of the small intestine. Gastric lipase, like other human lipases, possesses an amphiphilic peptide loop covering the active site like a lid or flap (Winkler et al. 1990) that undergoes conformational rearrangement when contact occurs with the lipid/water interface. Hence the hydrophobic portion of the loop binds to and is activated by the surface energy of the fat droplet allowing the active site to cleave a single fatty acid from the sn1/sn3 position of dietary fats (Carriere et al. 1993; Hamosh 1990). These fatty acid products are surface active, and hence associate with the oil–water interface modifying its composition and promoting further emulsification. This initial gastric lipolytic step is considered an important aspect in improving the efficiency of the subsequent hydrolysis of fats and oils by pancreatic lipase (Pafumi et al. 2002).

5.3.2 Effect of Emulsion Droplet Size

Oral processing allows any unemulsified ingested lipids to be delivered to the stomach in a crudely emulsified state. In comparison, fats and oils that are already finely emulsified within a food substrate are usually delivered with minimal changes to their droplet size distribution. Hence there can be considerable variation in the size range and surface area of fat droplets that enter the stomach, according to the structure and material properties of the food matrix being consumed.

In view of the specific action of gastric and intestinal lipases at the oil–water interface, it is likely that lipolytic efficiency is related to the available surface area, i.e. to the emulsion droplet size. This has been confirmed in an in vivo comparison of the rates of digestion of two intragastrically delivered emulsions of differing droplet size (a fine emulsion of ~ 0.7 μm and a coarse emulsion of ~ 10.1 μm) but equivalent interfacial composition (Armand et al. 1999). The rate of gastric lipolysis was found to be 1.7–3.3 times greater in the fine emulsion than the course emulsion 4 h after delivery, while ~ 14% of the original fine emulsion was hydrolysed to either fatty acids or diglycerides during the first half hour, a significantly greater proportion than that in coarse emulsions during the same time period. Similar findings have been obtained in vitro for fine emulsions stabilised with other emulsifiers (Borel et al. 1994; Pafumi et al. 2002).

Interestingly, the faster lipolysis of the fine emulsion was also accompanied by a fourfold to ninefold increase in the mean droplet size relative to that on delivery, this increase occurring principally in the first half hour. In contrast, the course emulsion displayed only a modest increase in droplet size. The findings led these workers to postulate that while fatty acids produced as a consequence of lipolysis may displace food amphiphiles in the original interfacial layer on account of their generally greater surface activity, they do not necessarily provide good interfacial stabilisation. Hence the weakening of the interfacial layer from orogenic displacement (i.e. the displacement of a less surface active interfacial component by a more surface active species) by fatty acids causes the droplets to be more prone to coalescence.

5.3.3 Emulsion Structuring in the Gastric Environment

Explorations of the correlation between the efficiency of gastric lipolysis and the surface area of the emulsion generally assume that interfacial composition does not vary between the emulsions under comparison. However, it is known that the sensitivity of the interfacial layer to gastric chemistry and biomechanics can greatly influence the stability of emulsions within the stomach, which may in turn influence lipolysis.

The acidic environment of the stomach can influence the stability of ingested emulsions by its effect on constituent emulsifying agents. Generally, food emulsions are stabilised by one of three classes of emulsifying ingredients: proteins, e.g. casein, whey, soy; non-protein biopolymers, e.g. propylene glycol alginate, octenylsuccinate starch, acetylated pectin; and polar lipids, e.g. lecithin, polysorbates, sodium stearoyl lactylate. These amphiphilic agents confer stability to oil droplets, not only by adsorbing onto any new interfaces formed during homogenisation and lowering their surface energy, but also by preventing their re-coalescence either by inducing a charge that causes electrostatic repulsion (ionic emulsifiers) or by steric hindrance, i.e. the physical obstruction of droplet coalescence.

Ionic emulsifiers can be particularly sensitive to changes in pH. For instance, proteinaceous polyionic emulsifiers lose their electrostatic properties at their isoelectric point (pI), i.e. the pH region of net neutral charge, causing the emulsion to become susceptible to flocculation. This phenomenon has been demonstrated in vitro using confocal microscopy. Model emulsion systems stabilised by the milk protein β-lactoglobulin become highly flocculated under simulated gastric conditions (Sarkar et al. 2009b). The pI of β-lactoglobulin is in the region of 4.7–5.2. The parent β-lactoglobulin emulsion has a charge of ~−60 mV, and hence is stable at a pH of 6.8. Exposure of the β-lactoglobulin emulsion to simulated gastric fluid lowers the pH of the emulsion to a point where is can no longer be stabilised by electrostatic repulsion forces, causing emulsion droplets to aggregate as a result of attractive van der Waals interactions. Proteinaceous emulsifiers show considerable variation in their isoelectric points, as this depends on their primary structure and the relative balance between differently charged amino acid groups.

Following their entry into the stomach, a number of emulsifying food proteins, notably those with a pI less than 7, transit through their pI and gain a net positive charge as the lumen pH approaches 1.9–2.0. Theoretically, the establishment of a positive charge on the interfacial layer of an emulsion in excess of 20 mV would be sufficient to provide effective electrostatic stabilisation. However, the acid conditions that are necessary to establish or maintain such a charge must be rapidly established on entry to the stomach before the emulsion destabilises. In reality, this is rarely the case (McLauchlan et al. 1989). There is generally a significant delay before acidic conditions become established, chiefly because of the buffering capacity of salivary bicarbonate systems. Moreover, the ionisation of cationic and anionic side chains of proteins, including those which stabilise emulsions, can cause them to act as buffering agents and retard the rate at which the gastric luminal pH falls. In vitro experimentation with a number of emulsion systems stabilised by various polyionic biopolymers with a pI between acid and neutral pH confirm this. Hence emulsion droplets stabilised with isolated wheat protein (IWP), whey protein isolate (WPI), sodium caseinate, acetylated pectin and the ionic surfactant sodium stearoyl lactylate (SSL), all show extensive aggregation of the component droplets on exposure to a simulated gastric environment.

The efficiency of proteinaceous and other emulsifiers may change as they themselves undergo digestion by other intragastric enzymes. This is illustrated by the destabilisation of a lactoferrin-stabilised emulsion system under simulated gastric conditions (Sarkar 2010). It could be expected that a cationic lactoferrin interface would provide ongoing emulsion stabilisation at low pH as its pI of ~8.5 lies outside the range of variation in gastric pH (2–7). However, ongoing measurement of zeta-potential showed that the net surface charge decreased during the period of exposure to gastric conditions causing flocculation and (limited) coalescence of emulsion droplets from insufficient electrostatic repulsion. The loss in surface charge was attributed to a gradual hydrolysis of the protein at the interface, cleavage by pepsin causing the hydrophilic (polar and charged) elements of the lactoferrin molecule to be released from the interface. While these experiments were carried out in the absence of gastric lipase, it would seem reasonable to suppose that protein-stabilised emulsions can generally be destabilised in the stomach by peptic hydrolysis of the interfacial protein layers. Loss of emulsion stability as a consequence of proteolytic detachment of charged or polar proteinaceous regions on the surface of constituent droplets contributes not only to their flocculation but may also promote their coalescence by changing the surface rheology and lowering the mechanical strength of the interfacial layer.

It is noteworthy that intragastric destabilisation and consequent flocculation of protein stabilised emulsions within the gastric lumen may be transient, changing with the return of the lumen pH to acidic fasting levels and with the effects of ongoing intragastric digestion, notably the action of lipase, and in the case of protein-stabilised emulsions, the action of pepsin (Macierzanka et al. 2009). This transience has been illustrated by a further study (Hur et al. 2009) in which confocal microscopy showed flocculation did not persist in emulsions stabilised either by sodium caseinate or by whey proteins after a 2-h exposure to a simulated gastric environment.

Emulsion systems stabilised by non-ionic (e.g. polysorbate emulsions), zwitterionic (e.g. lecithin and other phospholipids) or cationic (e.g. lactoferrin, lysozyme, chitosan) interfacial layers would be expected to show greater resistance to flocculation on exposure to gastric conditions (Reis et al. 2008b). For non-ionic emulsifiers, stability is primarily imparted by either Marangoni effects (stabilisation through surfactant mobility on the interface) or through steric stabilisation, and is therefore unrelated to either pH or ionic strength. In the case of emulsions stabilised by cationic emulsifiers, decreasing pH is actually more likely to improve stability against flocculation by progressively increasing net surface positive charge under more acidic conditions. Again, emulsions stabilised by non-protein surface active biopolymers, such as acetylated pectins, chitosan, propylene glycol alginates and modified celluloses, may behave differently as these agents are likely to be less sensitive to gastric conditions, notably the change to an acidic pH and especially the action of proteolytic enzymes. These agents may also provide steric hindrance to the binding of gastric lipase, although this hypothesis is currently unconfirmed (Mun et al. 2006).

Where gastric lipase is able to adsorb onto the oil–water interface, lipolysis is able to occur, and FFAs and diglycerides are formed. Diglycerides formed through

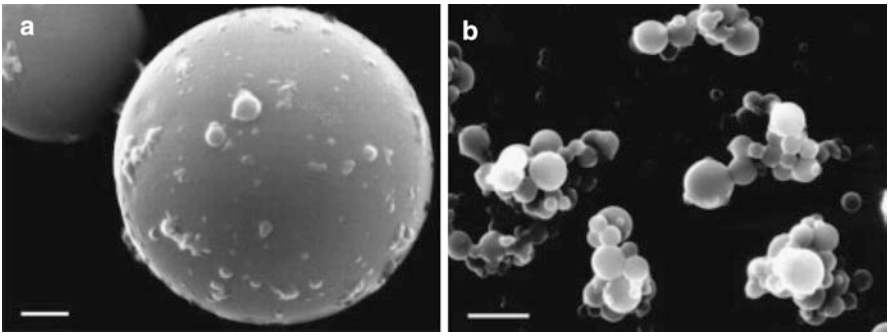


Fig. 5.2 Evolution of protonated fatty acids during digestion inhibits gastric lipase. **a** Nodules that appear on the surface of the emulsions during the digestion of triolein emulsions have been attributed to the formation of domains of lipolysed fatty acids. **b** The formation of such nodules is thought to bring about the fusion of droplets. Scale bar = 2 µm. (Pafumi et al. 2002)

lipolysis are still relatively apolar molecules and remain solubilised in the core of emulsion droplets. In contrast, FFAs are considerably more amphiphilic, particularly in the acidic gastric environment where they are protonated, and thus surface active. The accumulation of fatty acids at the droplet surface is believed to promote removal of existing interfacial components by orogenic displacement. Under certain conditions, fatty acids may accumulate in localised sites on the surface of emulsion droplets forming nodules (Fig. 5.2a) (Pafumi et al. 2002). As mentioned earlier, the surface energy of interfaces laden with fatty acids is relatively high and causes neighbouring droplets to coalesce, particularly in situations where droplet interfaces are in close contact, such as in a flocculated emulsion.

Scanning electron microscopy shows that the nodular regions are sites of apparent bridging and fusion between droplets (Fig. 5.2b). Such droplet associations may be driven by a local increase in surface energy due to the increased proportions of fatty acids at the interface. Hence, following exposure to a simulated gastric fluid, the range of droplet size in an emulsion was observed to increase from 0.5–2 to 2.6–6 µm (Pafumi et al. 2002). Given this mechanism, it might be expected that gastric lipolysis will cause any emulsion system to undergo some degree of coalescence, irrespective of the nature of the interface. However, a body of evidence suggests that emulsion structuring in the stomach is also influenced by the fatty composition in the lipid cores of the droplets. The lipids in most foods are almost entirely liquid at in-body temperatures and the contents of smaller droplets that are composed of such fats and oils are readily admixed as they coalesce. However, in emulsions that are composed of droplets that contain a proportion of fats that remain solid at body temperatures, the droplets undergo partial coalescence when they enter the gastric environment and irreversibly agglomerate, each component droplet maintaining some of its original form and composition (Golding and Wooster 2010). The extent of such agglomeration is greatly enhanced by flocculation (Golding and Wooster 2010), as when the component droplets are in close proximity, they are more prone to film rupture due to extensive film thinning between high numbers of associated

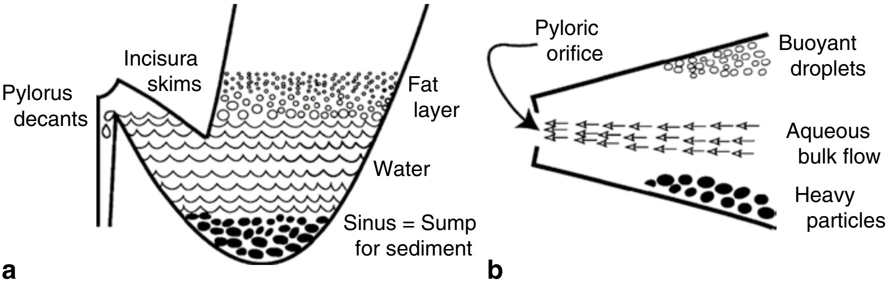


Fig. 5.3 Hypothetical implications of the configuration of the stomach at rest and during digestive activity on the disposition of its luminal contents. **a** Layering and phase discrimination. Contents layer in the vertical stomach according to their density. Particles (gravity of 1.2) settle in the sinus, fat (gravity of 0.9) floats on top, with watery contents in between. Watery contents fill the distal antrum and escape into the descending duodenum ahead of solid particles and fat. Small particles moving with the aqueous phase advance over the pyloric ridge into the duodenum (decanting), while fat is retained by the incisura (skimming). **b** Deflection of particles and fat droplets by the antral boundaries. The conical shape of the antrum with the pylorus at its upper end could act like an inverted funnel. During flow pulses, watery contents and tiny particles of water density make up the rapid core stream. Large particles and fat droplets move along the boundaries and are likely to be retained by the walls of the antrum. Sequential flow pulses would deposit gastric contents in the distal stomach according to their density gradients (Schulze 2006). See also Chap. 9

droplets. The flocculated droplets may then undergo heterogeneous coalescence, localised film rupture, and coalescence between neighbouring droplets, causing coalescence events to propagate into the surrounding droplets of the floc network.

Foods that undergo extensive partial coalescence in the gastric environment can form highly agglomerated structures with relatively low surface area. Compared with their fully coalesced counterparts, partially coalesced agglomerates are more resistant to biomechanical shear forces. Hence the formation of such structures may have important effects on the efficiency of gastric lipolysis, influencing not only the surface area that is available for population with molecules of gastric lipase but also the rate of transit of fats through the stomach (Fig. 5.3). Moreover, they may subsequently influence subsequent intestinal lipolysis.

Intragastric destabilisation of emulsions through flocculation or coalescence can also lead to the formation of a floating cream or oil layer on the surface of the stomach contents. When the subject is in the sitting or standing position, this layer is at some distance from the pylorus and will empty from the stomach only after the oil depleted watery layer beneath it has exited (Fig. 5.3). Conversely, more stable emulsion systems and those of higher viscosity are more homogeneously dispersed throughout the contents of the stomach, and hence will empty form it in a more uniform fashion (Marciani et al. 2006; Schwizer et al. 2006).

The persistence of a floating layer of oil within the stomach, as observed on MRI (Fig. 5.4), indicates that any shear forces developed within the gastric fundus are insufficient to maintain or re-establish an emulsion. Moreover, the heterogeneity in the intragastric distribution of lipids appears to influence the physiology of gastric emptying, i.e. the watery lipid-depleted residue of the unstable emulsion exits the

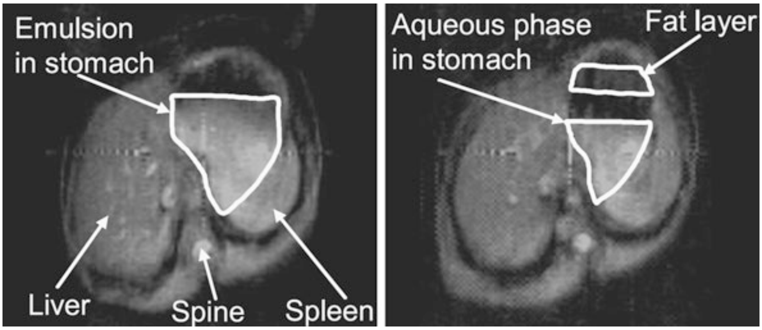


Fig. 5.4 The contrast between fat and water on MRI enables mapping of the spatial distribution of food components within the stomach in a supine subject. Emulsions that are stable to the acidic conditions within the stomach show a uniform distribution of the fatty phase throughout the stomach cavity *(left-hand image)*. An emulsion that is sensitive to stomach acid is seen to break forming a layer of oil on the top of the contents *(right-hand image)*. (Marciani et al. 2007)

stomach more promptly than does a stable, intact emulsion (Marciani et al. 2007). The relatively low concentration of lipids in the former may generate less physiological feedback inhibition of gastric emptying when it enters the small intestine than would the entry of an intact emulsion (Marciani et al. 2007). This hypothesis is supported by the observation that the rate of emptying of an unstable emulsion is reduced to a greater extent than that of a stable emulsion towards the end of gastric emptying, i.e. at the time when the coalesced layer of lipid exits the stomach (Marciani et al. 2007). This mechanism is further evidenced by the fact that emulsions that are unstable in the gastric environment generally generate lower levels of circulating cholecystokinin (CCK) than do stable emulsion, and hence are less effective at suppressing hunger than those that are stable (Foltz et al. 2009).

The contribution of gastric motility to the (re)establishment of oil-in-water emulsions within the chyme is a matter of some debate. It has been suggested that the mixing forces generated as a result of antropyloral muscular contractions may aid fat re-emulsification when elements in the contained digesta are highly surface active, e.g. contain significant levels of fatty acids (Armand et al. 1993, 1994; Carey et al. 1983). However, recent quantification of the forces generated within the distal stomach (compression force of 0.53–0.78 N and shear rates ~1–6 s^{-1}) (Marciani et al. 2007) suggests that the forces generated during trituration are too weak to emulsify free oil, at least via the classical high shear homogenisation mechanism favoured by some (Lentle et al. 2010; Marciani et al. 2001). Hence oral emulsification may provide the principal means by which free dietary lipids are crudely emulsified.

While there may be some intragastric dispersion of these crude emulsions facilitated by the generation of fatty acids, it is increasingly evident that the weak gastric forces may actually contribute more to the coalescence of unstable emulsions than to their further dispersion. As discussed earlier, this is especially likely when emulsions spontaneously flocculate in the gastric environment. Thus the relative stability of the emulsified state within the stomach may depend upon the outcome of concurrent intragastric proteolysis and lipolysis, as well as the presence of endogenous and exogenous surface active agents and their resistance to intragastric conditions. Accordingly, there may be pronounced variations in particle size and surface area of emulsions that transit to the small intestine after gastric processing.

5.4 The Intestinal Processing of Fats and Oils

5.4.1 Basic Processes

The bulk of fat digestion and the assembly of the products of digestion of lipids for absorption occur in the small intestine. The hydrolysis of remaining triglycerides is mediated via the combined action of human gastric lipase (HGL) and co-lipase-dependant human pancreatic lipase (HPL) (Armand 2007; Lowe 2002; Mu and Høy 2004). HPL hydrolyses the sn1 and sn3 positions of dietary triglycerides producing FFAs and 2-monoglycerides. It has a broad pH activity range (4.5–7.5) with optimal activity at around pH 6.5 (Carriere et al. 1993). Structural rearrangement of 2-monoglyerides can occur during digestion and subsequent lipolysis can complete the degradation of triglycerides to glycerol and constituent FFAs. Approximately 25% of fatty acids at the sn2 position undergo such isomerisation (Akesson et al. 1976). The activity of HPL may be inhibited by the presence of amphiphiles at the interface of emulsions, whereas this phenomenon does not occur with HGL (Bernbäck et al. 1989, 1990). However, HPL activity is optimised in the presence of co-lipase and bile salts (Bläckberg et al. 1979).

Co-lipase is a non-enzymatic protein co-factor with a molecular weight of ~10 kDa (Erlanson-Albertsson 1992). It facilitates the action of HPL by assisting in its adsorption onto emulsion interfaces and stabilising the ‘lid domain’ of the enzyme in the open position (Lowe 1997). Co-lipase is an amphiphilic protein with a ‘three-finger’ topology, the tips of each finger containing the most hydrophobic regions of the protein. Co-lipase binds to the C-terminal end of pancreatic lipase, in such a position that the three fingers are oriented outwards from the complex. The projecting fingers, together with the lid region of HPL, form a continuous hydrophobic domain, which increases the ability of the complex to locate at the oil–water interface.

Conjugated bile salts secreted by the liver via the biliary tree, along with phospholipids also secreted by the liver, are necessary for both the digestion and absorption of lipids (Bauer et al. 2005; Donovan et al. 1993). Conjugated bile salts have a flat planar structure, the common steroidal nucleus forming the hydrophobic side, and the hydroxyl groups and ionic head group forming the hydrophilic side. While they share a common backbone, bile salts exhibit a range of hydrophobicities as a result of the different head groups (glycine or taurine) with which the bile acids are conjugated, and the number and position of hydroxyl groups. Bile salts can both aid and inhibit pancreatic lipolysis. In vitro experiments have shown that the high surfactancy of bile salts sterically hinders the adsorption of HPL onto emulsion interfaces (Wickham et al. 1998). However, this inhibitory effect is overcome by the presence of co-lipase, the co-lipase/HPL complex being able to absorb onto a bile salt-covered interface (Lowe 1997, 2002).

In many cases, the high surfactancy of bile salts actually aids digestion as they are able to bring about orogenic displacement of inhibiting exogenous surfactants and allow binding of co-lipase (Maldonado-Valderrama et al. 2008). Small molecule surfactants, such as polyoxyethylene sorbitan mono-oleate (Tween 80), can inhibit HPL, even in the presence of co-lipase (Gargouri et al. 1983). However, when present in concentrations above their critical micellar concentration (CMC, 2–4 mM) bile salts can displace Tween 80 and other surfactants from oil–water interfaces allowing the co-lipase/HPL complex to bind to the bile salt-coated interface, thereby facilitating lipolysis. As lipolysis proceeds, fatty acids and monoglycerides tend to build up at the emulsion interface. Both reaction products have high surface activity and would tend to displace both gastric lipase and pancreatic lipase from the interface (Pafumi et al. 2002; Reis et al. 2008a; Reis et al. 2008b). However, such inhibition is prevented by bile salts, and to lesser extent phospholipids, removing these accumulating products from the interface by solubilising them in mixed micelles in the bulk aqueous phase (Mu and Høy 2004; Porter et al. 2007). Hence the continuing uptake of bile salt into the lipid interface facilitates further lipid digestion. The partition (Madenci and Egelhaaf 2010) of lipolytic products into mixed micelles completes the intraluminal stage of digestion of lipid droplets (Mu and Høy 2004).

The equilibrium between the assembly and dissolution of mixed micelles (see below) may allow a sufficient concentration of fatty acids to be maintained in the aqueous phase at the surface of small intestinal enterocytes to allow their efficient absorption by diffusive and active transport (Abumrad and Storch 2006). The complex biophysics that govern this process are in turn affected by a number of environmental factors, including the effects of local changes in ionic strength and pH (Madenci and Egelhaaf 2010), and the local concentrations and rate of absorption of different bile salts (Dawson et al. 2006).

5.4.2 The Influence of Emulsion Structure on Small Intestinal Digestion

As with gastric lipolysis, intestinal lipolysis occurs at the oil–water interface, a process that requires the maintenance of a colloidal state. If lipolytic efficiency is limited by available surface area, then it should increase with decreasing particle size and with increasing specific surface area. In vitro studies indicate that the rate of pancreatic lipolysis does increase with increasing specific surface area, at least for emulsions with common interfacial composition (Seimon et al. 2009). In vivo studies using direct intraduodenal delivery, so as to preclude any changes to the composition or structure of the emulsion in more proximal compartments of the gut, allow direct comparisons to be conducted between emulsions of differing structure and stability. Recently, such a comparison of the physiological effects of a series of duodenally delivered emulsions of differing droplet size (0.26–170 µm) but similar composition (Seimon et al. 2009) confirmed that emulsions of the smallest droplet size induced statistically higher levels of secretion of CCK and peptide YY, with higher concurrent levels of plasma triglycerides also being recorded. Variations in surface area of directly delivered emulsions have also been reported to influence satiety and food intake (Maljaars et al. 2008).

However, studies in which emulsions of differing droplet size are ingested normally yield conflicting results. The initial droplet size of ingested rather than directly delivered emulsions is reported to have only limited influence on the rate of lipid uptake in the small intestine (Golding and Wooster 2010). This finding fits in with our knowledge of the varying effects of the gastric environment on different emulsion systems and indicates that the size of the emulsion entering the small intestine is dictated by interposing processes. This finding is also supported by other work showing that the uptake of vitamins A and E that were incorporated into two different sized emulsions (0.7 vs 10 µm) did not differ significantly following normal ingestion (Borel et al. 2001).

As discussed earlier, bile salts and phospholipids are known to displace existing surface active material from interfacial layer of the emulsion droplets providing access for the co-lipase complex. For example, bile salts are able to displace β-lactoglobulin at both air–water and oil–water interfaces (Maldonado-Valderrama et al. 2009; Maldonado-Valderrama et al. 2008). However, the ease with which bile salts are able to locate on the interface varies with its physical structure and the characteristics of the surfactant that is being displaced. Common amphiphiles, such as proteins and other biopolymers, are generally readily displaced by bile salts. However, emulsions with more complex surface structures, such as those with multilayered interfaces formed by electrostatic associations, may present a more significant physical barrier to bile salt adsorption at the oil–water interface, and hence retard the rate and extent of pancreatic lipolysis. The latter is exemplified by studies of emulsions formed by electrostatic interfacial complexation between an anionic form of the surfactant lecithin and the cationic biopolymer chitosan (Mun et al. 2006; Park et al. 2007), which showed a slower rate of in vitro pancreatic lipolysis relative to an emulsion stabilised solely by lecithin. This was attributed to the formation of a thicker interfacial layer with pronounced cationic characteristics.

Other phenomena operating at the oil–water interface, such as crystallisation, denaturation (thermal, high pressure), and enzymatic or chemical cross-linking (transglutaminase/gluteraldehyde), and the presence of particulates could all potentially influence its structural and mechanical properties, and inhibit bile salt adsorption. For example, the reduction of pancreatic lipolysis in vitro by a number of galactolipids (notably digalactosyldiacylglycerol) is thought to result from their resisting orogenic displacement by bile salts. As a consequence, the galactolipids indirectly reduce the ability of co-lipase and lipase to bind to the droplet surface, thereby delaying and reducing lipolysis (Chu et al. 2009).

Conversely, recent work exposing surface active proteins in emulsions to thermal treatments in order to increase their resistance to orogenic displacement by bile salts indicates that improving the rheological strength of the interfacial layer through protein cross-linking is not necessarily sufficient to increase the ability of the protein to resist displacement (Sandra et al. 2008). Even in cases where the interfacial layer of an ingested emulsion has been demonstrated to resist displacement by bile salts, the structural integrity of such an interface may be degraded by prior exposure to the gastric environment.

The results of investigations into the dissolution of emulsions in the luminal environment of the small intestine are equivocal. Ongoing lipolysis of droplets with movement of fatty acid products into mixed micelles could be envisaged to lead to a reduction in the size of the droplets and their eventual disappearance from the emulsion. Indeed, an overall reduction in droplet size and density of four synthetic emulsion systems stabilised either by protein (sodium caseinate or whey) or other surfactants (Tween 80 or lysolecithin) has been demonstrated in vitro after a 4-h exposure to small intestinal secretions (Hur et al. 2009). However, exposure of emulsions stabilised by proteinaceous, non-proteinaceous and lipid-based amphiphiles in vitro to simulated small intestinal secretions in stirred media brought about an initial increase in droplet diameter through coalescence, regardless of the composition of the original interface (Golding and Wooster 2010; Sarkar et al. 2010). It is noteworthy that the shear conditions in the latter study may not have matched those in the small intestine, at least during the postprandial period when segmentation is induced by nutrients in the chyme (notably the presence of decanoic acid) (Gwynne et al. 2004). Nevertheless, this result is supported by an in vivo study of duodenal aspirates showing no overall reduction in droplet size following the administration of lecithin-stabilised droplets, either as fine or coarse emulsions (Armand et al. 1999).

A number of studies indicate that the physical characteristics of fats in the dispersed phase of an emulsion may impede lipolysis and the formation of mixed micelles. For instance, the rate and extent of pancreatin-induced lipolysis were reported to be higher in an emulsion of liquid fat droplets maintained at body temperature than in an emulsion containing solid fat that was maintained under similar conditions (Bonnaire et al. 2008). Lipolysis was also substantially impeded in an emulsion system that contained 25% of fat that was solid at 37 °C and which underwent extensive agglomeration under gastric conditions compared with that of a gastrically stable emulsion composed of entirely liquid droplets (Fig. 5.5).

In some situations, intestinal hydrolysis of triglyceride substrates that are liquid at 37 °C may generate products that are solid at body temperature and whose subsequent metabolism or absorption is impaired. For instance, this may occur when the monoglyceride formed following cleavage of the fatty acids in the sn1 and sn3 positions has a lower melting point than the original triglyceride from which it was formed. Thus a slower metabolism has been observed for chylomicrons composed of triglycerides with an OSO (i.e. oleic-stearic-oleic in sn1, sn2 and sn3) esterification sequence relative to those containing triglycerides with the sequence OOS (oleic-oleic-stearic) (Redgrave et al. 1988), both triglycerides being entirely liquid at in-body temperature. This phenomenon is attributed to the fact that the melting point of 2-monostearin produced from OSO is about 60 °C while that of

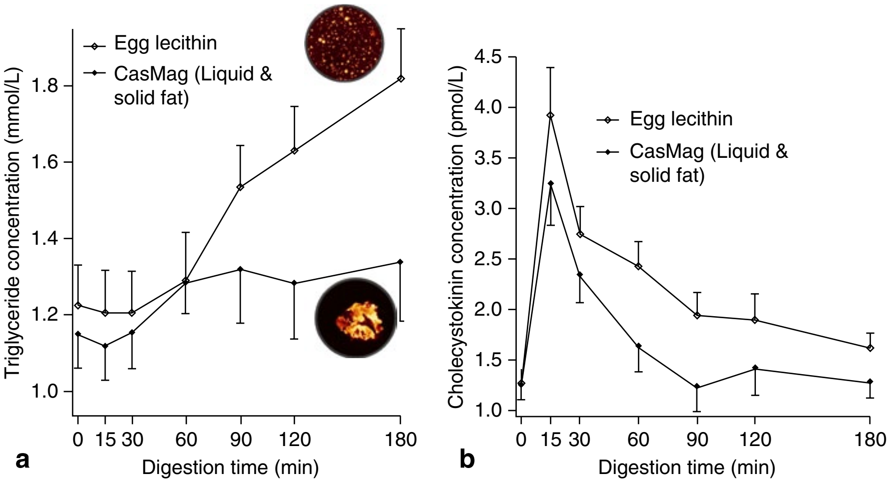


Fig. 5.5 Variation in plasma TAG (**a**) and CCK levels (**b**) following ingestion of two types of emulsion containing 30% fat. The emulsion of egg lecithin is known to be gastric stable, while the CasMag emulsion is considered to undergo partial coalescence in the gut (the *inset confocal images* show the differing gastric behaviour of emulsions under in vitro conditions). The differences in plasma-TAG uptake are thought to result from the considerably lower surface area of the partially coalesced emulsion

2-monoolein produced from OOS is liquid at in-body temperatures. Hence the formation of a highly crystalline 2-monostearin interfacial layer during emulsion digestion may cause displacement or exclusion of bile salts, and lipase enzymes and co-factors, resulting in the slowing of OSO lipolysis relative to OOS. However, the possibility of manipulating lipid hydrolysis through triglyceride fractionation and regio-specificity is rendered complex and occasionally contradictory due to the highly diverse composition of food oils (Kubow 1996).

5.5 Mixed Micelle Formation and Transport of Lipids Across the Lumen, Mucin Layer and Epithelium

While certain lipids, e.g. cholesterol, may be directly and actively absorbed by enterocytes (Chen 2001), triglycerides must be hydrolysed and their components absorbed. The process of absorption is mediated by the association of the fatty acids and partial glycerides that are produced by lipolysis at the oil–water surfaces of droplets with bile acids and phospholipids in mixed micelles. A number of lipids other than fatty acids, including cholesterol and fat-soluble vitamins, may also associate with these structures (Dawson et al. 2006).

Mixed micelles are thought to transport fatty acids and monoglycerides from the oil–water interface of the emulsion droplet across the lumen space to the mucosa, where their ambient concentrations are estimated to increase by a factor of around 1,000 (Hofmann 1999). While mixed micelles diffuse more slowly than their constituent monomers, the local increase in concentration of fatty acids within the micelle itself accelerates diffusion at least one hundredfold (Hofmann 1999). This increase in diffusion rate may help to prevent any restriction of the rapid protein-facilitated transport of longer-chain fatty acids into the enterocyte (Abumrad and Storch 2006).

5.5.1 The Movement of Lipids from Droplets to Micelles

The dynamics that govern the uptake of lipolytic products from emulsion droplets into micelles are not completely understood. The propensity of amphiphilic bile salts to rapid 'self-assembly' above a CMC and their ability to form into hierarchical series of structures according to local ionic concentration and pH (Madenci and Egelhaaf 2010) may aid in the transport of lipids and lipolytic products to the enterocyte. Thus low pH and high bile salt concentrations within the hepatobiliary system favour the aggregation of particular bile acids into rod-like micelles (Lopez et al. 1996). In the lumen of the small intestine, the components of these structures readily integrate with lipolytic products while the active absorption of bile by the enterocytes lowers ambient bile salt concentrations adjacent to the mucosa, which may favour micellar dissociation, and solution of lipolytic products.

The dissolution of emulsion droplets into mixed micellar entities was thought until recently to be a surface mediated effect that resulted from the accumulation of a number of highly surface active species at the oil–water interface, namely bile salts, monoglycerides and phospholipids. The high concentration of surfactants at the droplet surface was thought to allow fatty acids to be incorporated into micelles or vesicles, which subsequently dissociated from the droplet surface. Such a process would expose fresh sites on the droplet surface for subsequent attachment of HPL and lipolysis (Porter et al. 2007). More recently, observations on model lipid systems have indicated that polar lipids formed during lipolysis may bring about mesophase structuring of the interior of the droplet, which may promote its dissolution. However, given the wide variation in the fatty acid compositions of the various food oils, it seems unlikely that such internal mesophase structures could consistently be formed.

Recent work indicates that the transition between the various forms of self-assembled micelle and micro-emulsion is influenced not only by their composition, i.e. the relative proportions of the fatty acid and monoglyceride products of the hydrolysis of triglycerides, but also by pH. Hence reversible transition of liquid crystalline forms (hydrodynamic radius 120 nm) of mixtures of oleic acid and monooleate to vesicles (60 nm) occurs at a pH between 7–8 (Salentinig et al. 2010), i.e. the luminal pH of the ileum, where a significant proportion of dietary lipids are absorbed (Lin et al. 1996).

5.5.2 The Mucus Layer and Lipid Transport to the Epithelium

The mucus layer that coats the intestinal epithelium is known to act as a barrier to the transit of soluble nutrients (see Chap. 10) either by charge exclusion, by size exclusion or by limiting advection, i.e. by creating an ‘unstirred water layer’ that lengthens the mean molecular diffusion path. The permeability of mucus to mixed biliary micelles is reported to be reduced with respect to that of an unstirred water layer of similar thickness (Hernell et al. 1990; Staggers et al. 1990; Wiedmann et al. 2001), a reduction as low as 25% having been found by one group (Wiedmann et al. 2001). This small difference may result from the tightly ordered structure of the micelles, with polar lipids arranged radially with their hydrophilic heads facing outwards and the bile acids arranged perpendicularly with their hydrophilic faces facing outwards (Hjelm et al. 1995). Such a structure may be subject to a degree of ionic repulsion from charged elements on mucin side chains but such repulsion would prevent interaction of the hydrophobic surfaces of bile acids with localised intensely hydrophobic regions in the mucoprotein backbones.

Neutron-scattering studies indicate that the mixed micelles formed in bile from bile acids, phosphatidylcholine and cholesterol, and those formed in the intestine from bile acids, fatty acids and monoglycerides have a similar primary molecular arrangement with an overall cylindrical form (Hjelm et al. 1995). These primary forms are known to aggregate into more structurally complex and liquid crystalline aggregates. Moreover, the liquid crystalline, helical and disc-like forms are known to dissociate into primary forms as bile salt concentration declines (Madenci and Egelhaaf 2010). If similar complex structures persist at the lumen periphery and are governed by the same dynamics, then these intestinal micelles will disintegrate into their primary cylindrical forms as local concentrations of bile decline from active absorption by the enterocytes. Such a process may help to circumvent any volume exclusion effect from mucin to the passage of large micelles.

5.5.3 Absorption by Enterocytes

Currently, it is unclear whether the bulk of absorption of the products of lipolysis results from endocytosis of intact micelles or from a dynamic equilibrium of the contents with the surrounding aqueous media, which may allow absorption by specific transporters in the apical membranes of enterocytes or by passive solution in the phospholipid bilayer of the apical plasma membrane.

The absorption of fatty acids by saturatable transporters in the apical membrane (membrane associated fatty acid binding protein FABPpm, fatty acid transport proteins FATP1 or fatty acid translocase CD6) (Abumrad and Storch 2006) and the concurrent local lowering of concentrations of bile acids by similarly situated sodium-dependent bile acid transporters (Oelkers et al. 1997) could avoid any need for the permeation of intact micelles through the apical membrane of the enterocyte and the close approach of micelles to the apical membrane, which is necessary for endocytosis (Gonnella and Neutra 1984). The efficiency of any process of absorption that is mediated by the aqueous phase would still be dependent upon the extent to which micelles are able to penetrate the overlying unstirred water layer. Specifically, the absorption of soluble fats would be lowered in proportion to the thickness of the unstirred water layer intervening between the micelle and the apical membranes of enterocytes (Wilson et al. 1971).

Recent evidence supports the hypothesis that fatty acids, monoglycerides (Hansen et al. 2007) and other lipid-soluble materials (Hansen et al. 2009) are directly endocytosed via scavenging proteins in clathrin-coated pits between adjacent microvilli (Hansen et al. 2003) (see Chap. 10). The mechanism may involve a ‘flip-flop’ transmucosal transfer mechanism (Stahl 2004) rather than a conventional carrier-mediated transport directly through the membrane. Such a flip-flop mechanism could involve transient incorporation of FFAs into the outer portion of the lipid bilayer in a clathrin-coated pit leading to increases in the curvature of the membrane at this site and the local promotion of invagination, i.e. endocytosis (Hansen et al. 2009).

The colonic absorption of short-chain fatty acids differs from that described above. Fatty acids of up to six carbon atoms length, commonly termed ‘short-chain fatty acids’, are thought to be in ionised form as a result of the low pH within the colonic lumen (Chu and Montrose 1996) and are thus readily soluble in water and are absorbed by ion-linked exchangers on the apical surfaces of enterocytes, notably SCFA-Cl and SCFA-HCO_3 (Jørgensen et al. 1998), to be metabolised therein or to transit via elements of the vascular system to the liver via the portal vein. While solubility decreases with increase in chain length necessitating the need for micellar solubilisation, medium-chain fatty acids with hydrocarbon chains of up to 11 still possess limited solubility, which may accordingly affect their assembly, transport and uptake (van Aken 2010).

5.5.4 Other Dietary Factors Influencing Absorption via Their Effects on Micelles

The structure and properties of the mixed micellar systems may be influenced by a number of dietary components. High concentrations of calcium can impede the formation of mixed micelles by binding to hydrolysed fatty acids, or to bile salts (Bendsen et al. 2008; Gueguen and Pointillart 2008). The presence of dietary plant sterols and sterol esters (phytosterols) appears to reduce the enterohepatic circulation of cholesterol by competitive inhibition of its absorption into mixed micelles (Mel’nikov et al. 2004) leading to its excretion rather than (re)absorption. The latter indicates that similar competitive dynamics may operate in the absorption of various hydrophobic molecules from oil droplets in emulsions.

5.6 Lipids in the Colon and Rectum

Up to 95% of the fatty acids contained in dietary lipids are absorbed within the small intestine; hence very little fat normally enters the colon. While the short-chain fatty acids that are produced by enzymatic or microbial digestion can readily be absorbed by colonic enterocytes, non-hydrolysed triglycerides cannot be absorbed at this site and do not undergo significant microbial digestion. Fat-soluble vitamins that are not absorbed when digestive disorders affect the proximal segments of the gut may partition into the fat and transit the colon without undergoing absorption or bacterial degradation. Malabsorption of fat-soluble micronutrients can occur in a similar manner following the consumption of lipase inhibitors or non-digestible lipid mimetics, such as sucrose polyesters, which are commonly used to reduce fat intake.

Fat is generally not found in an emulsified state in the lower bowel as the bulk of bile acids have either been absorbed in the ileum (Dawson et al. 2006) or have undergone deconjugation by colonic commensal microflora. Undigested lipid in colonic digesta generally comprises an oily liquid, which when present in significant amounts softens the stool and lowers its density, causing it to float in the lavatory pan. The presence of liquid fat in the distal colon commonly results in anal leakage, presumably due to the less efficient recto-anal obturation when the rectum is distended with liquid (see Chap. 8).

5.7 Future Directions

The dispersion of liquid fat into a colloidal state is critical for the efficient digestion of lipids. Common dietary items contain fats and oils of remarkably diverse composition and in various physical states, ranging from finely homogenised and stable emulsions such as milk, through aggregated and agglomerated structures such as ice cream, to the unemulsified fats and oils found in fried and baked foods. It appears that, for the most part, the body is able to regulate the state of these diverse structures during their transit through the proximal gastrointestinal tract to achieve a degree of emulsification that is sufficient for their efficient digestion. Hence the human gut can digest and absorb around 95% of dietary triglycerides. However, the efficiency of lipid digestion and the means to impair it are of increasing interest given the critical role of fats in the genesis of obesity and the other components of the metabolic syndrome. The formulation of foods that impair the efficiency of lipid digestion may provide a means of combating such disorders. The manipulation of the physical states of dietary lipids may provide a useful strategy in this regard. However, such manipulations must pay due heed to any adverse effects such as the impairment of absorption of fat-soluble nutrients and undesirable presence of lipid in the colon.

An increased understanding of the manner in which the physical structures of dietary lipids and lipids in chyme influence their digestion may also provide opportunities for improving the efficiency of lipid digestion. Such improvements may be beneficial in the nutrition of infants and the elderly, as well those with impaired digestion of lipids. Such benefits may pertain not only to augmenting the absorption of lipids but also to enhancing the absorption of lipid-soluble micronutrients. It is becoming increasingly apparent that we have the potential to control the physical structures of dietary lipids. This opens up the possibility of delivering tailored emulsion and lipid structures that follow a specific digestive pathway and provide a targeted physiological benefit.

Our increasing understanding of the manner in which proteins orient across the oil–water interface, and the manner in which polar and apolar elements of these proteins are subject to physical and enzymatic degradation has important connotations, not only for the process of digestion and absorption, but also for our understanding of the vascular transport of lipids. It is generally accepted that the viscoelastic behaviour of proteins located at the oil–water interface is critical to their ability to stabilise emulsions (Weinberg et al. 2000). Apolipoprotein A-IV, a surface active protein involved in the vascular transport of lipid droplets, appears to have evolved a molecular conformation with mobile amphipathic helices, which move in or out of the oil–water interface in response to changes in surface pressure, and hence can adapt to changes in interfacial area. Currently, it is not known whether any proteins contained in intestinal secretions have similar properties. At all events, the existence of such steriochemically adaptive molecular behaviour illustrates that it may be possible to steriochemically ‘redesign’ oligopeptides with important physiological functions, such as insulin. This redesign could aim to produce a molecule that is oriented across the oil–water interface of dietary emulsions so as to avoid proteolytic digestion, opening the way to oral delivery in a pharmaceutical context.

References

Abumrad N, Storch J (2006) Role of membrane and cytosolic fatty acid binding proteins in lipid processing by the small intestine. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Academic Press, San Diego, pp 1693-1709

Adams S, Singleton S, Juskaitis R, Wilson T (2007) In-vivo visualisation of mouth-material interactions by video rate endoscopy. Food Hydrocolloids 21:986-995

Akesson B, Gronowitz S, Herslöf B (1976) Stereospecificity of hepatic lipases. FEBS Lett 71:241-244

Armand M (2007) Lipases and lipolysis in the human digestive tract: Where do we stand? Curr Opin Clin Nutr Metabol Care 10:156-164

Armand M, Borel P, Dubois C, Senft M, Peyrot J, Salducci J, Lafont H, Lairon D (1993) Physicochemical properties of emulsions and lipolysis of dietary-fat in the human stomach. Gastroenterology 104:A608

Armand M, Borel P, Dubois C, Senft M, Peyrot J, Salducci J, Lafont H, Lairon D (1994) Characterization of emulsions and lipolysis of dietary lipids in the human stomach. Am J Physiol 266:G372-381

Armand M, Pasquier B, Andre M, Borel P, Senft M, Peyrot J, Salducci J, Portugal H, Jaussan V, Lairon D (1999) Digestion and absorption of 2 fat emulsions with different droplet sizes in the human digestive tract. Am J Clin Nutr 70:1096-1106

Bakker J, Mela DJ (1996) Effect of emulsion structure on flavor release and taste perception. In: McGorrin RJ, Leland JV (eds) Flavour-food interactions. ACS Publications, pp 36-47

Bauer E, Jakob S, Mosenthin R (2005) Principles of physiology of lipid digestion. Asian-Australasian J Anim Sci 18:282-295

Bellamy M, Godinot N, Mischler S, Martin N, Hartmann C (2009) Influence of emulsion composition on lubrication capacity and texture perception. Int J Food Sci Tech 44:1939-1949

Bendsen NT, Hother AL, Jensen SK, Lorenzen JK, Astrup A (2008) Effect of dairy calcium on fecal fat excretion: A randomized crossover trial. Int J Obes 32:1816-1824

Bernbäck S, Bläckberg L, Hernell O (1989) Fatty acids generated by gastric lipase promote human milk triacylglycerol digestion by pancreatic colipase-dependent lipase. Biochim Biophys Acta 1001:286-293

Bernbäck S, Bläckberg L, Hernell O (1990) The complete digestion of human milk triacylglycerol in vitro requires gastric lipase, pancreatic colipase-dependent lipase, and bile salt-stimulated lipase. J Clin Invest 85:1221-1226

Bläckberg L, Hernell O, Bengtsson G, Olivecrona T (1979) Colipase enhances hydrolysis of dietary triglycerides in the absence of bile salts. J Clin Invest 64:1303-1308

Bonnaire L, Sandra S, Helgason T, Decker EA, Weiss J, McClements DJ (2008) Influence of lipid physical state on the in vitro digestibility of emulsified lipids. J Agric Food Chem 56:3791-3797

Borel P, Armand M, Ythier P, Dutot G, Melin C, Senft M, Lafont H, Lairon D (1994) Hydrolysis of emulsions with different triglycerides and droplet sizes by gastric lipase in vitro. Effect on pancreatic lipase activity. J Nutr Biochem 5:124-133

Borel P, Pasquier B, Armand M, Tyssandier V, Grolier P, Alexandre-Gouabau MC, Andre M, Senft M, Peyrot J, Jaussan V (2001) Processing of vitamin A and E in the human gastrointestinal tract. Am J Physiol 280:G95-103

Carey MC, Small DM, Bliss CM (1983) Lipid digestion and absorption. Annu Rev Physiol 45:651-677

Carriere F, Laugier R, Barrowman JA, Douchet I, Priymenko N, Verger R (1993) Gastric and pancreatic lipase levels during a test meal in dogs. Scand J Gastroenterol 28:443-454

Chen HC (2001) Molecular mechanisms of sterol absorption. J Nutr 131:2603

Chu BS, Rich GT, Ridout MJ, Faulks RM, Wickham MSJ, Wilde PJ (2009) Modulating pancreatic lipase activity with galactolipids: Effects of emulsion interfacial composition. Langmuir 25:9352-9360

Chu S, Montrose MH (1996) Non-ionic diffusion and carrier-mediated transport drive extracellullar pH regulation of mouse colonic crypts. J Physiol 494:783-793

Dawson PA, Shneider BL, Hofmann AF (2006) Bile formation and the enterohepatic circulation. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Academic Press, San Diego, pp 1437–1462

de Bruijne DW, Bot A (1999) Fabricated fat-based foods. In: Rosenthal AJ (ed) Food texture: Measurement and perception. Aspen, Gaithersburg, pp 185-227

de Bruijne DW, Hendrickx HACM, Alderliesten L, de Looff J (1993) Mouthfeel of foods. In: Dickinson E, Walstra P (eds) Food colloids and polymers: Stability and mechanical properties. Royal Society of Chemistry, Cambridge, pp 204-213

Dickinson E (1996) Biopolymer interactions in emulsion systems: Influences on creaming, flocculation, and rheology. In: Parris N, Kato A, Creamer LK, Pearce J (eds) Macromolecular interactions in food technology ACS Publications, pp 197-209

Donovan JM, Jackson AA, Carey MC (1993) Molecular species composition of inter-mixed micellar/vesicular bile salt concentrations in model bile: Dependence upon hydrophilic-hydrophobic balance. J Lipid Res 34:1131-1140

Dresselhuis DM, De Hoog EHA, Cohen Stuart MA, Van Aken GA (2008a) Application of oral tissue in tribological measurements in an emulsion perception context. Food Hydrocolloids 22:323-335

Dresselhuis DM, De Hoog EHA, Cohen Stuart MA, Vingerhoeds MH, Van Aken GA (2008b) The occurrence of in-mouth coalescence of emulsion droplets in relation to perception of fat. Food Hydrocolloids 22:1170-1183

Dresselhuis DM, Stuart MAC, van Aken GA, Schipper RG, de Hoog EHA (2008c) Fat retention at the tongue and the role of saliva: Adhesion and spreading of 'protein-poor' versus 'protein-rich' emulsions. J Colloid Interface Sci 321:21-29

Erlanson-Albertsson C (1992) Pancreatic colipase. Structural and physiological aspects. Biochim Biophys Acta 1125:1-7

Fahey D, Small DM (1986) Surface properties of 1,2-dipalmitoyl-3-acyl-sn-glycerols. Biochemistry 25:4468-4472

Fahey D, Small DM (1988) Phase behavior of monolayers of 1,2-dipalmitoyl-3-acyl-sn-glycerols. Langmuir 4:589-594

Foltz M, Maljaars J, Schuring EAH, van der Wal RJP, Boer T, Duchateau GSM, Peters HPF, Stellaard F, Masclee AA (2009) Intragastric layering of lipids delays lipid absorption and increases plasma CCK but has minor effects on gastric emptying and appetite. Am J Physiol 296:G982-991

Gargouri Y, Julien R, Bois AG, Verger R, Sarda L (1983) Studies on the detergent inhibition of pancreatic lipase activity. J Lipid Res 24:1336-1342

Glantz PO (1997) Interfacial phenomena in the oral cavity. Colloid Surf A Physicochem Eng Aspects 123:657-670

Golding M, Wooster TJ (2010) The influence of emulsion structure and stability on lipid digestion. Curr Opin Colloid Interface Sci 15:90-101

Gonnella PA, Neutra MR (1984) Membrane-bound and fluid-phase macromolecules enter separate prelysosomal compartments in absorptive cells of suckling rat ileum. J Cell Biol 99:909-917

Gueguen L, Pointillart A (2008) Digestive and metabolic interactions between lipids and calcium. Sciences des aliments 28:117-127

Gwynne RM, Thomas EA, Goh SM, Sjovall H, Bornstein JC (2004) Segmentation induced by intraluminal fatty acid in isolated guinea-pig duodenum and jejunum. J Physiol 556:557-569

Hamilton JA, Miller KW, Small DM (1983) Solubilization of triolein and cholesteryl oleate in egg phosphatidylcholine vesicles. J Biol Chem 258:12821-12826

Hamilton JA, Small DM (1981) Solubilization and localization of triolein in phosphatidylcholine bilayers: A 13 C NMR study. Proc Natl Acad Sci USA 78:6878-6882

Hamosh M (1990) Lingual and gastric lipases. Nutrition 6:421-428

Hansen GH, Niels-Christiansen LL, Immerdal L, Danielsen EM (2003) Scavenger receptor class B type I (SR-BI) in pig enterocytes: Trafficking from the brush border to lipid droplets during fat absorption. Gut 52:1424-1431

Hansen GH, Niels-Christiansen LL, Immerdal L, Nystrom BT, Danielsen EM (2007) Intestinal alkaline phosphatase: Selective endocytosis from the enterocyte brush border during fat absorption. Am J Physiol 293:G1325-1332

Hansen GH, Rasmussen K, Niels-Christiansen LL, Danielsen EM (2009) Endocytic trafficking from the small intestinal brush border probed with FM dye. Am J Physiol 297:G708-715

Hernell O, Staggers JE, Carey MC (1990) Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. Biochemistry 29:2041-2056

Hjelm RP, Thiyagarajan P, Schteingart C, Hofmann AF, Alkan-Onyuksel H, Ton-Nu H-T (1995) Structure of mixed micelles present in bile and intestinal contents based on studies of model systems. In: Hofmann AF, Paumgartner G, Stiehl A (eds) Bile acids in gastroenterology; basic and clinical advances. Kluwer Academic, Dordrecht, pp 41-58

Hofmann AF (1999) Bile acids: The good, the bad, and the ugly. News Physiol Sci 14:24-29

Hur SJ, Decker EA, McClements DJ (2009) Influence of initial emulsifier type on microstructural changes occurring in emulsified lipids during in vitro digestion. Food Chemistry 114:253-262

Hutchings JB, Lillford PJ (1988) The perception of food texture - the philosophy of the breakdown path. J Text Stud 19:103-115

Jørgensen J, Holtug K, Jeppesen PB, Mortensen PB (1998) Human rectal absorption of short-and medium-chain C2-C10 fatty acids. Scand J Gastroenterol 33:590-594

Kodali DR, Atkinson D, Small DH (1989) Molecular packing in triacyl-sn-glycerols: Influences of acyl chain length and unsaturation. J Disp Sci Techn 10:393-440

Kubow S (1996) The influence of positional distribution of fatty acids in native, interesterified and structure-specific lipids on lipoprotein metabolism and atherogenesis. J Nutr Biochem 7:530-541

Lentle RG, Janssen PWM, Goh K, Chambers P, Hulls C (2010) Quantification of the effects of the volume and viscosity of gastric contents on antral and fundic activity in the rat stomach maintained *ex vivo*. Dig Dis Sci DOI 10.1007/s10620-010-1164-y:1-12

Lin HC, Zhao XT, Wang L (1996) Fat absorption is not complete by midgut but is dependent on load of fat. Am J Physiol 271:G62-67

Lopez F, Samseth J, Mortensen K, Rosenqvist E, Rouch J (1996) Micro-and macrostructural studies of sodium deoxycholate micellar complexes in aqueous solutions. Langmuir 12:6188-6196

Lowe ME (1997) Structure and function of pancreatic lipase and colipase. Annu Rev Nutr 17:141-158

Lowe ME (2002) The triglyceride lipases of the pancreas. J Lipid Res 43:2007-2016

Macierzanka A, Sancho AI, Mills ENC, Rigby NM, Mackie AR (2009) Emulsification alters simulated gastrointestinal proteolysis of β-casein and β-lactoglobulin. Soft Matter 5:538-550

Madenci D, Egelhaaf SU (2010) Self-assembly in aqueous bile salt solutions. Curr Opin Colloid Interface Sci 15:109-115

Maldonado-Valderrama J, Gunning AP, Ridout MJ, Wilde PJ, Morris VJ (2009) The effect of physiological conditions on the surface structure of proteins: Setting the scene for human digestion of emulsions. Eur Phys J E 30:165-174

Maldonado-Valderrama J, Woodward NC, Gunning AP, Ridout MJ, Husband FA, Mackie AR, Morris VJ, Wilde PJ (2008) Interfacial characterization of β-lactoglobulin networks: Displacement by bile salts. Langmuir 24:6759-6767

Maljaars PWJ, van der Wal RJR, Haddeman EA, Peters HPF, Beindorff C, Masclee AAM (2008) Effect of droplet size of a fat emulsion delivered in the small intestine on satiety and food intake in healthy volunteers. Eur J Gastroenterol Hepatol 20:A54

Malone ME, Appelqvist IAM, Norton IT (2003) Oral behaviour of food hydrocolloids and emulsions. Part 1. Lubrication and deposition considerations. Food Hydrocolloids 17:763-773

Marciani L, Gowland PA, Fillery-Travis A, Manoj P, Wright J, Smith A, Young P, Moore R, Spiller RC (2001) Assessment of antral grinding of a model solid meal with echo-planar imaging. Am J Physiol 280:G844-849

Marciani L, Wickham M, Singh G, Bush D, Pick B, Cox E, Fillery-Travis A, Faulks R, Marsden C, Gowland PA (2007) Enhancement of intragastric acid stability of a fat emulsion meal delays gastric emptying and increases cholecystokinin release and gallbladder contraction. Am J Physiol 292:G1607-1613

Marciani L, Wickham MSJ, Bush D, Faulks R, Wright J, Fillery-Travis AJ, Spiller RC, Gowland PA (2006) Magnetic resonance imaging of the behaviour of oil-in-water emulsions in the gastric lumen of man. Br J Nutr 95:331-339

McClements DJ, Decker EA, Park Y (2009) Controlling lipid bioavailability through physicochemical and structural approaches. Crit Rev Food Sci Nutr 49:48-67

McLauchlan G, Fullarton GM, Crean GP, McColl KE (1989) Comparison of gastric body and antral pH: A 24 hour ambulatory study in healthy volunteers. Br Med J 30:573-578

Mel'nikov SM, Seijen ten Hoorn JWM, Eijkelenboom APAM (2004) Effect of phytosterols and phytostanols on the solubilization of cholesterol by dietary mixed micelles: An in vitro study. Chem Phys Lipids 127:121-141

Mela DJ, Langley KR, Martin A (1994) Sensory assessment of fat content: Effect of emulsion and subject characteristics. Appetite 22:67-81

Mu H, Høy CE (2004) The digestion of dietary triacylglycerols. Prog Lipid Res 43:105-133

Mun S, Decker EA, Park Y, Weiss J, McClements DJ (2006) Influence of interfacial composition on in vitro digestibility of emulsified lipids: Potential mechanism for chitosan's ability to inhibit fat digestion. Food Biophysics 1:21-29

Norton I, Fryer P, Moore S (2006) Product/process integration in food manufacture: Engineering sustained health. AIChE J 52:1632-1640

Norton I, Moore S, Fryer P (2007) Understanding food structuring and breakdown: Engineering approaches to obesity. Obes Rev 8:83-88

Oelkers P, Kirby LC, Heubi JE, Dawson PA (1997) Primary bile acid malabsorption caused by mutations in the ileal sodium-dependent bile acid transporter gene (SLC10A2). J Clin Invest 99:1880-1887

Pafumi Y, Lairon D, de la Porte PL, Juhel C, Storch J, Hamosh M, Armand M (2002) Mechanisms of inhibition of triacylglycerol hydrolysis by human gastric lipase. J Biol Chem 277:28070-28079

Park GY, Mun S, Park Y, Rhee S, Decker EA, Weiss J, McClements DJ (2007) Influence of encapsulation of emulsified lipids with chitosan on their in vivo digestibility. Food Chemistry 104:761-767

Porter CJH, Trevaskis NL, Charman WN (2007) Lipids and lipid-based formulations: Optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov 6:231-248

Redgrave TG, Kodali DR, Small DM (1988) The effect of triacyl-sn-glycerol structure on the metabolism of chylomicrons and triacylglycerol-rich emulsions in the rat. J Biol Chem 263:5118-5123

Reis P, Miller R, Kragel J, Leser M, Fainerman VB, Watzke H, Holmberg K (2008a) Lipases at interfaces: Unique interfacial properties as globular proteins. Langmuir 24:6812-6819

Reis P, Miller R, Leser M, Watzke H, Fainerman VB, Holmberg K (2008b) Adsorption of polar lipids at the water- oil interface. Langmuir 24:5781-5786

Rossetti D, Yakubov GE, Stokes JR, Williamson AM, Fuller GG (2008) Interaction of human whole saliva and astringent dietary compounds investigated by interfacial shear rheology. Food Hydrocolloids 22:1068-1078

Salentinig S, Sagalowicz L, Glatter O (2010) Self-assembled structures and pKa value of oleic acid in systems of biological relevance. Langmuir 26:11670-11679

Sandra S, Decker EA, McClements DJ (2008) Effect of interfacial protein cross-linking on the in vitro digestibility of emulsified corn oil by pancreatic lipase. J Agric Food Chem 56:7488-7494

Sarkar A (2010) Behaviour of milk protein-stabilized oil-in-water emulsions in simulated physiological fluids. Riddet Institute. Massey university, Palmerston North

Sarkar A, Goh KKT, Singh H (2009a) Colloidal stability and interactions of milk-protein-stabilized emulsions in an artificial saliva. Food Hydrocolloids 23:1270-1278

Sarkar A, Goh KKT, Singh RP, Singh H (2009b) Behaviour of an oil-in-water emulsion stabilized by [beta]-lactoglobulin in an *in vitro* gastric model. Food Hydrocolloids 23:1563-1569

Sarkar A, Horne DS, Singh H (2010) Pancreatin-induced coalescence of oil-in-water emulsions in an in vitro duodenal model. Int Dairy J 20:589-597

Schulze K (2006) Imaging and modelling of digestion in the stomach and the duodenum. Neurogastroenterol Mot 18:172-183

Schwizer W, Steingoetter A, Fox M (2006) Magnetic resonance imaging for the assessment of gastrointestinal function. Scand J Gastroenterol 41:1245-1260

Seimon RV, Wooster T, Otto B, Golding M, Day L, Little TJ, Horowitz M, Clifton PM, Feinle-Bisset C (2009) The droplet size of intraduodenal fat emulsions influences antropyloroduodenal motility, hormone release, and appetite in healthy males. Am J Clin Nutr 89:1729-1736

Silletti E, Vingerhoeds MH, Norde W, van Aken GA (2007) The role of electrostatics in saliva-induced emulsion flocculation. Food Hydrocolloids 21:596-606

Singh H, Ye A, Horne D (2009) Structuring food emulsions in the gastrointestinal tract to modify lipid digestion. Prog Lipid Res 48:92-100

Staggers JE, Hernell O, Stafford RJ, Carey MC (1990) Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 1. Phase behavior and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human beings. Biochemistry 29:2028-2040

Stahl A (2004) A current review of fatty acid transport proteins (SLC27). Pflügers Arch 447:722-727

van Aken GA (2010) Relating food emulsion structure and composition to the way it is processed in the gastrointestinal tract and physiological responses: What are the opportunities? Food Biophysics DOI 10.1007/s11483-010-9160-5

van Aken GA, Vingerhoeds MH, de Hoog EHA (2007) Food colloids under oral conditions. Curr Opin Colloid Interface Sci 12:251-262

Vingerhoeds MH, Blijdenstein TBJ, Zoet FD, van Aken GA (2005) Emulsion flocculation induced by saliva and mucin. Food Hydrocolloids 19:915-922

Vingerhoeds MH, Silletti E, de Groot J, Schipper RG, van Aken GA (2009) Relating the effect of saliva-induced emulsion flocculation on rheological properties and retention on the tongue surface with sensory perception. Food Hydrocolloids 23:773-785

Weinberg RB, Cook VR, DeLozier JA, Shelness GS (2000) Dynamic interfacial properties of human apolipoproteins A-IV and B-17 at the air/water and oil/water interface. J Lipid Res 41:1419-1427

Wickham M, Garrood M, Leney J, Wilson PDG, Fillery-Travis A (1998) Modification of a phospholipid stabilized emulsion interface by bile salt: Effect on pancreatic lipase activity. J Lipid Res 39:623

Wiedmann TS, Herrington H, Deye C, Kallick D (2001) Analysis of the diffusion of bile salt/phospholipid micelles in rat intestinal mucin. Chem Phys Lipids 112:81-92

Wilson FA, Sallee VL, Dietschy JM (1971) Unstirred water layers in intestine: Rate determinant of fatty acid absorption from micellar solutions. Science 174:1031-1033

Winkler FK, d'Arcy A, Hunziker W (1990) Structure of human pancreatic lipase. Nature 343:771-774

Chapter 6
The Physical Characteristics of Digesta

Contents

6.1 Introduction

To understand how digestion and absorption are optimised or impeded by the physical properties of digesta, it is necessary to know how they may influence flow, mixing, transit and fractionation in the various compartments of the gut. Such an understanding may aid in the design of foods that reduce the efficiency of absorption of abundant nutrients, such as glucose and cholesterol (Dikeman and Fahey 2006), from the human diet or in the design of feeds that increase the digestive efficiency of farm stock (Buxton and Redfearn 1997; McClements et al. 2008).

The absorption of nutrients from the lumen of the mammalian intestine would be unduly slow in the absence of mechanisms that bring about macroscopic mixing (Crank 1975; Penry and Jumars 1986). Absorption by diffusion alone would require greatly prolonged digesta residence times given the relatively large cross-sectional area of the lumen of most gut components (France et al. 1993).

R. G. Lentle, P. W. M. Janssen, *The Physical Processes of Digestion*,
DOI 10.1007/978-1-4419-9449-3_6,

The intensity of small intestinal motility, and hence the extent of mixing of contained digesta, are known to be influenced both directly and indirectly by the concentration of particular nutrients within the lumen (Borgstrom and Arborelius 1975; Defilippi and Gomez 1995; Gwynne et al. 2004; Huge et al. 1995; Schemann and Ehrlein 1986; Siegle and Ehrlein 1988). However, recent work shows that, in some species at least, this motility is relatively insensitive to changes in the physical properties of digesta, notably its apparent viscosity (Lentle et al. 2007). Such insensitivity is likely to render the efficiency of small intestinal mixing vulnerable to the effects of viscogenic dietary components. This may account for the reduction in digestive efficiency (Hetland and Svihus 2001) and in the rate of absorption of nutrients (Cameron-Smith et al. 1994; Reppas and Dressman 1992; Wood et al. 1994) when animals are maintained on intrinsically viscous diets such as ground wheat (Hetland and Svihus 2001), or on diets that have been supplemented with indigestible viscous components such as guar gum (Roberts et al. 1989). While such effects may be small, they may have a significant influence on the long-term energy budgets of animals that are competing for resources (Sibley and Calow 1986). Moreover, they may influence feed conversion efficiency in animal production, e.g. poultry (Hetland et al. 2004).

Currently, our knowledge of the general physical properties of digesta is incomplete. Digesta consists of solid and liquid phases, and at times a gaseous phase. Moreover, evidence suggests the solid and liquid phases may move relative to each other during transit, i.e. phase separation occurs. Hence the physical interactions between these various phases are complex. A limited number of papers describe these interactions in digesta. However, the physical processes that govern such interactions are well described in other particulate systems. Thus we consider evidence from a range of particulate systems, including that obtained from digesta where available.

We begin this chapter by considering the nature of fluid flow and mixing. We examine in detail the physical properties of particle suspensions and how these impact flow and mixing. Subsequently, we focus on the phase separation of semisolid digesta and the effect of a gaseous phase.

6.2 Physics of Flow and Mixing

The flow of fluid through a contained space may be orderly or turbulent depending upon the relative magnitudes of the viscous and inertial forces within it. This relationship is characterised by the Reynolds number (Re), which is defined as the ratio of the inertial to the viscous forces. When inertial forces dominate, the Reynolds number will be high and individual elements of the fluid will have sufficient kinetic energy to form eddies of widely differing length, duration and direction, i.e. turbulent mixing will be possible. When viscous forces predominate, the Reynolds number will be low, and the movement of fluid will be constrained to orderly layers, the flow regime being termed laminar.

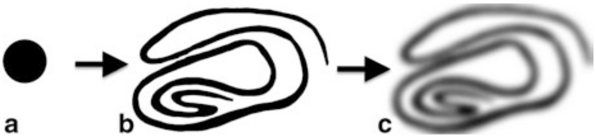


Fig. 6.1 Representation of the processes occurring during the mixing of fluids. **a** For this example, one fluid is initially shown as a droplet. **b** Eddies and vortices cause the fluids to be stretched and folded resulting in the formation of striations. **c** Interdiffusion of fluid components generates mixing at a microscale. (After Ottino 1989)

The mixing of fluids can be thought of as a process of repeated stretching and folding to create layers or striations within it (Ottino 1989) (Fig. 6.1). The comparatively slow process of molecular diffusion will then operate between the striations to attain a uniform concentration. In a turbulent flow regime, the chaotic eddies are very effective at stretching and folding the fluid in widely different length scales, and hence there is less need for molecular diffusion to attain a uniform concentration. However, as the viscosity of a fluid increases, it is necessary to increase the rate of flow of the fluid to impart sufficient inertial forces to maintain the Reynolds number and to generate turbulence. At very high viscosities, this is energetically expensive.

At low Reynolds numbers, the flow of a viscous fluid through a long straight cylindrical tube follows the Hagen–Poiseuille law and can be viewed as occurring in a series of concentric cylindrical lamina with the flow in each lamina having a velocity determined only by its radial distance from the centre of the tube. Mixing efficiency in such a regime is poor as it is largely reliant on molecular diffusion. However, the gastrointestinal tract is convoluted, the wall exhibits a complex pattern of active and passive longitudinal and radial movements, and the flow of digesta is pulsatile. All these factors can generate vortices and sequences of stretching and folding that serve to mix the fluid contents.

The flow regime in a pipe affects the residence time distribution (RTD) of the fluid traversing it (Levenspiel 1999). This phenomenon has been used to investigate the flow regime of fluid in ex vivo segments of the ileum of the brushtail possum (Janssen et al. 2007). The RTD obtained with watery perfusates showed that the contractile activity of the segment was able to induce turbulence while the RTD obtained with viscous perfusate (1% guar gum) indicated that the contractile activity was capable of engendering considerable mixing in the absence of turbulence. In this book, we refer to the latter as vortical flow, where a vortex is a fluid motion having a closed or spiralling streamline. The picture that emerges is that flow regime, and modes of mixing and mass transfer vary according to the location along the gut and the rheology of the contained digesta. Hence, when the luminal contents have low viscosity, turbulence is possible and can generate extensive mixing. However, as the digesta moves distally and becomes more viscous, it is increasingly energetically expensive to maintain such turbulence and efficient mass transfer must depend on alternative less energy-intensive strategies.

6.3 Physical Properties of Fluid Digesta

Fluid digesta consists of a suspension of particulate matter in a liquid phase which, as it transits the succession of gastrointestinal segments distal to the stomach, becomes increasingly less fluid as a result of the progressive absorption of water (Binder 1983). Consequently, the behaviour of digesta changes progressively from that of a pseudoplastic fluid whose properties are governed by the suspending liquid and the concentration, shape, size and buoyancy of the particulate matter, to that of a particulate aggregate whose properties are governed by the strength, plasticity, elasticity, cohesion and permeability of the solid elements.

We shall discuss the effects of these parameters on the physical properties of digesta in a broadly similar sequence. In this section, we consider the effects arising in more dilute suspensions from the interaction of individual particles with and within the liquid phase. In the subsequent section, we examine more concentrated systems of particles and the effects arising from the presence of gaseous and oil phases.

6.3.1 The Liquid Phase of Digesta

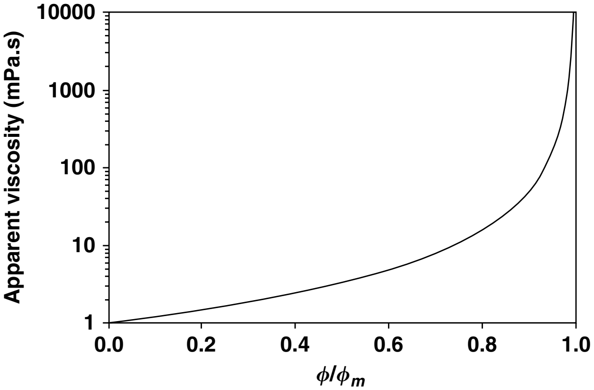
The viscosity of the liquid phase of digesta has been determined over a range of shear rates in only a few species (Dikeman and Fahey 2006). These limited data include the liquid phase of caecal digesta from the pig (Takahashi and Sakata 2004), of ileal digesta from the dog (Dikeman et al. 2007), and the caecal digesta of chickens (Takahashi et al. 2004). The liquid phase from digesta of each of these species exhibited low viscosity and Newtonian behaviour when the animals were maintained on natural diets. The viscosity of Newtonian fluids is independent of shear rate as the rate of movement between adjacent layers of fluid is proportional to the applied force. Hence the results of experiments in which the viscosity of the liquid phase is determined at high and unphysiological rates of shear will nevertheless be valid at the low rates of shear that are found in the digestive tract.

The presence of significant amounts of dissolved macromolecules, such as non-digestible polysaccharides, may increase the apparent viscosity of the liquid phase of digesta and impart shear-thinning behaviour (pseudoplasticity), i.e. cause the apparent viscosity to decrease with increase in shear rate (Barnes et al. 1989). Thus the addition of 4% carboxymethylcellulose to the diet of pigs is reported to increase the apparent viscosity of the liquid phase of digesta along the entire intestine and to render it pseudoplastic (McDonald et al. 2001). In this case, if the apparent viscosity of the liquid phase of digesta is determined at shear rates that are higher than those found in the digestive tract, its magnitude will be significantly lower than that in vivo.

6.3.2 Viscosity of Whole Digesta

While the viscosity of the liquid phase of digesta is generally low and Newtonian, that of whole digesta is considerably higher and also varies with shear rate in a non-

Fig. 6.2 Relationship between apparent viscosity of a suspension and solids volume fraction *(ϕ)* relative to the maximum packing fraction *(ϕ_m)*. (Krieger and Dougherty 1959)



Newtonian manner. Hence, whole digesta from a number of species is reported to be much more viscous and non-Newtonian (Dikeman et al. 2007; Takahashi et al. 2004; Takahashi and Sakata 2004), presumably as a result of the particulate matter content.

The apparent viscosity of a suspension is usually expressed as a function of the ratio of the solids volume fraction *(ϕ)* to the maximum packing fraction *(ϕ_m)* (Barnes et al. 1989; Krieger and Dougherty 1959) (Fig. 6.2). In dilute suspensions, i.e. low ϕ, there is little interaction between the particles and any increase in apparent viscosity results largely from hydrodynamic effects in which the streamlines of the liquid phase diverge around each particle during flow (Barnes et al. 1989). Hence the viscosity of a dilute particulate suspension is determined principally by the viscous properties of the liquid phase, with a small correction for ϕ. If the liquid phase in which the particles are suspended behaves in a Newtonian manner, a dilute suspension of particles will behave similarly.

The contribution of particle–particle interactions to overall viscosity generally increases with the concentration of particles, i.e. ϕ, while the contribution from the liquid phase decreases (Barnes et al. 1989). The magnitude of particle–particle interactions is governed by a number of factors, including the degree of friction and lubrication between them, and the ease with which they become entangled (Coussot and Ancey 1999). The probability of particles becoming entangled increases with their length (Doi and Edwards 1986; Ferry 1980), and the probability of entanglement of long narrow particles, such as are commonly found in some digesta, increases with their concentration (Doi and Edwards 1986; Ferry 1980). Colloidal forces or Brownian motion may also contribute to the interaction of particles when their diameters are below 1 µm. The relative influence of these factors is likely to vary with the type of food ingested.

The large differences between the viscosity of whole digesta and that of its liquid phase, that have been reported in a number of species (Dikeman et al. 2007; Takahashi et al. 2004; Takahashi and Sakata 2004), indicate that digesta should be classified as dense suspensions. This classification is also consistent with its reported non-Newtonian behaviour, specifically pseudoplasticity, found in whole digesta sampled from several points along the gut of the brushtail possum (Lentle

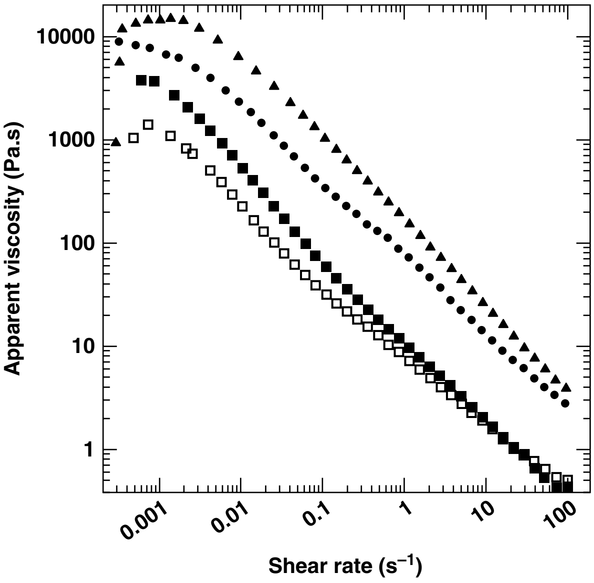


Fig. 6.3 Flow curves of digesta from the proximal small intestine *(open square)*, distal small intestine *(solid square)*, caecum *(circle)* and colon *(triangle)* of the brushtail possum. (Reproduced from Lentle et al. 2005)

et al. 2005) (Fig. 6.3). The pseudoplasticity is thought to result from the formation of microstructures by local aggregation of constituent particles, the extent of these structures varying with the level of shear. In essence, a dynamic balance is established between the rate at which particles interact within the suspension and form microstructures and the rate at which these structures are eroded by shear; a high shear rate lowering the average size of microstructures, and hence lowering the apparent viscosity (Stickel and Powell 2005). If non-spherical particles are present in the suspension, a balance is established between the tendency of the particles to become aligned with the direction of flow at higher shear rates and the randomisation of directional orientation induced by Brownian motion at lower shear rates (Barnes et al. 1989).

Although the volume fraction of the solid phase *(ϕ)* is known to be important in determining the behaviour of particulate suspensions under shear, its mathematical relationship with the rheological behaviour of mammalian digesta has not yet been investigated. Indeed, neither the volume fraction nor the maximum packing faction of the particulate matter in digesta have been accurately determined. This may in part be due to the difficulty of measuring ϕ, because it includes the volume of any liquid phase that is physically trapped within the particles.

If we assume that the dry matter content (DMC) is roughly correlated with ϕ, we may crudely assess the extent to which particle–particle interactions influence the apparent viscosity of mammalian digesta. Both the apparent viscosity (Lentle et al. 2005) and DMC (Hecker and Grovum 1975) of whole digesta increase as digesta traverses the length of the gut. The DMC of porcine digesta roughly doubles, increasing from 12.5 to 28.5% between the caecum and the rectum, while the apparent viscosity increases 45-fold (McRorie et al. 2000). Thus the apparent viscosity increases disproportionately to DMC and is likely to have significant effects on the flow and mixing of digesta.

While the DMC of digesta can provide some indication of the manner in which the proportion of particulate matter varies during the process of digestion, it is important to note the limitations of this approach. Digesta frequently contains particles consisting of hollow matrices with thin walls and substantial water-filled cavities, such as the lignified cell walls of vegetable matter that remain after their contents have been digested (Mandalari et al. 2008). These particles may occupy a high volume but have a low DMC. In this case, DMC gives little quantitative indication of ϕ and may under certain conditions vary independently of it, such as when water is expressed from the hollow cavities.

6.3.3 Effects of the Relative Size and Shape of Particles

The fragmentation of food by chewing is probabilistic in nature and creates a polydisperse distribution of particle sizes (Lucas 2004). There are practical limits in the size to which food particles may be reduced by mastication and gastric trituration (Lucas 2004). The division of smaller particles is energetically less efficient than division of larger particles as it is more dependent on plastic deformation than cracking (Lucas 2004). Rather than augmenting the speed of digestion by increasing surface area of the substrate (Lucas 2004), excessively fine particle sizes may adversely affect digestion. For instance, the feeding of finely ground wheat (geometric mean diameter, 300 µm) to chickens causes marked slowing of their growth (Amerah et al. 2007). Digesta that is composed principally of finer sized particles may also give less tactile stimulation to the mucosa. Hence the finer grinding of bran reduces its laxative effects (Heller et al. 1980).

While the transit of food particles larger than 1 mm from the stomach to the small intestine is restricted (Meyer et al. 1981), this restriction is relative and there are finite possibilities that larger particles may enter the small intestine (Itoh et al. 1986). Consequently, while physiological mechanisms may operate to retain and concentrate particles within certain size ranges in particular segments of the intestine, the size distribution of digesta generally remains polydisperse. A highly polydisperse suspension can generally pack more tightly, producing a greater ϕ_m, than will a monodisperse suspension. As a result, the apparent viscosity of a polydisperse suspension is generally lower at a given ϕ.

Suspensions of non-spherical particles have higher viscosities at a given ϕ than suspensions of spherical particles (Mewis and Macosko 1993). Therefore, alterations in the predominant shape of digesta particles may influence the flow of digesta, particularly in gut segments in which ϕ is high. Although little comparative data are available regarding the degree of variation in the shape or aspect ratio of digesta particles (Lirette et al. 1990; Robertson et al. 1992), this is likely to depend largely on the level of structural anisotropy in the common dietary items (Vincent 1990). The entanglement of longer particles is thought to be responsible for the selective retention of the solid relative to the liquid phase in the human terminal ileum (Hammer et al. 1993; Hebden et al. 1998).

6.4 Physical Properties of Semisolid Digesta

In fluid digesta, particulate aggregates do not span the volume of the lumen. Conversely, in a 'swallow-safe' food bolus and in the digesta found in the distal large bowel, particulate aggregates may become contiguous across the entire volume imparting predominantly solid-like characteristics, i.e. rendering it viscoelastic (Steffe 1996). Different strategies are required to digest and extract any nutrients contained within such a structure.

The modes of operation of the various gut compartments that handle fluid digesta have been likened to those of the continuous-flow stirred tank reactors (CSTRs) and plug flow reactors (PFRs) familiar to chemical engineers (Penry and Jumars 1986). The efficient operation of both reactor types require the contents to be uniformly and thoroughly mixed, which is best achieved by a turbulent regime. The establishment of a similar turbulent mixing regime in viscoelastic digesta would require greater energy expenditure to disrupt the more extensive particulate aggregates, an expenditure that may be unsustainable when the potential nutrient yield from the contained digesta is low (Lentle et al. 2002). Strategies for the digestion of nutrients in semisolid digesta appear to combine a less energy-intensive, laminar vortical regime with fractionation of the components, e.g. the separation and selective processing of fine nutrient-rich particles.

6.4.1 The Influence of Particle–Particle Interactions on Flow: Viscoelasticity

Whole digesta has viscoelastic properties, i.e. exhibits both solid-like and liquid-like behaviour. Studies of the dynamic rheology of the digesta of the brushtail possum showed that the elastic modulus (G′) (an index of solid-like behaviour) consistently exceeded the viscous modulus (G″) (an index of the tendency to flow) over a range of oscillation frequencies (Lentle et al. 2005). Further, the ratio between these two moduli increased along the gastrointestinal tract although continuing to remain at less than an order of magnitude. These findings indicate that the particulate elements of the digesta aggregated with locally transient (Ross-Murphy 1995) relaxable physical interactions to form weak gels (Lentle et al. 2005; Martinez-Ruvalcaba et al. 2007; Ross-Murphy 1995). Characteristically, such weak gels are able to rapidly reform their interacting particulate structures following their disruption by large deformation (Ross-Murphy 1995).

A material with a yield stress will not continuously change its shape when it is subjected to a given stress, and is termed a solid (Barnes et al. 1989). There is some uncertainty as to whether digesta requires a force that is sufficient to disrupt the particle structures within it before it will flow, i.e. displays a yield stress (Lentle et al. 2005; Takahashi and Sakata 2002). However, this doubt appears to reflect the general rheological debate regarding the existence of yield stresses in particulate suspensions (Barnes 1999). From a practical perspective, the most distal digesta (i.e. the stool) of a number of species appear to exhibit a yield stress over shear rates and time scales that are relevant to the digestive tract. For example, the force required to induce extrusion of digesta in a texture analyser increases in samples taken from various points along the porcine colon in a pattern that indicates that an appreciable yield stress develops around the mid transverse colon (McRorie et al. 1998). In rheological terms, the most distal digesta with a yield stress can best be described as a viscoelastic solid, while more proximal digesta would be classified as a viscoelastic liquid. The magnitude of the yield stress of a particulate aggregate is related to interparticle forces, particle volume fraction (ϕ), and the individual properties of particles (Poslinski et al. 1988). In general, an appreciable yield stress will only become apparent at higher values of ϕ (Zhou et al. 2001), i.e. in digesta with high DMC. Similarly, the magnitude of the yield stress will increase in digesta with smaller particle size (Zhou et al. 2001) and will be higher when particles are longer and thinner, i.e. more fibrous (Bennington et al. 1990).

The strengths of particulate aggregates are likely to vary with the type of food consumed, as a result of changes in the size, shape and composition of particles, and the interparticle forces. The application of a load that is sufficient to exceed the yield stress of the particulate material may cause a contiguous network of particles to deform permanently. Alternatively, the application of a lesser load may cause an elastic particle, or network of solid elastic particles, to deform while under load but to retain the capacity to regain its original shape once the load is removed, i.e. behave elastically. The elasticity of a suspension containing a contiguous network of digesta fibres will vary with ϕ, as well as with the source of the fibres and the extent of digestion, e.g. the extent that the cellulose/lignin ratio is altered by bacterial cellulolysis. An expression has been developed for the elasticity of a particulate suspension assuming that this results solely from the contiguous network of particles (Tatsumi et al. 2002). This assumption is likely to be valid for digesta that is formed from a natural diet, but may not adequately describe the situation where the liquid phase of digesta contains a high concentration of soluble non-digestible polysaccharides resulting in some viscous resistance to flow of the liquid phase (da Silva and Rao 1999). The changes in the elastic modulus of whole digesta are important in a number of digestive scenarios that are discussed later in the section on fluid expression.

6.4.2 *Faecal Water*

Water is held in association with the fibrous particulate matter of faeces in three ‘phases’ (Eastwood et al. 1983): water that is bound to hydrophilic surfaces of individual fibres; water that is held in the spaces between the fibres of the matrix; and water that is trapped within the cellular structures of the fibres. If the trapped water is restricted from exiting fibres during compression, it may increase the rigidity of fibres hindering matrix collapse, i.e. maintain interparticle voids.

Trapped faecal water may also act to sequester toxins, such as cytotoxic bile acids (Nagengast et al. 1995; Van Munster et al. 1993), as well as conveying them to the lignified surfaces of fibres with which they will bind (Eastwood 2007; Eastwood and Hamilton 1968). Further, some unfermented soluble fibres, such as those contained in psyllium seed, may effectively sequester water in unfermentable gels. These resist expression and promote bulk slippage and laxation (Marlett et al. 2000), thereby reducing compression of any other fibrous elements in the stool.

6.4.3 Effects of a Gaseous Phase in Particulate Aggregates

There is significant in situ formation of bubbles in colonic digesta from gas produced by the fermentative action of various enteral microflora on food residues such as non-starch polysaccharides (Suarez et al. 1997; Suarez and Levitt 2000). This can occur quite promptly in humans at times when the supply of fermentative substrates to the colon is suddenly increased following evacuation of the contents of the distal small intestine, notably immediately following the consumption of a meal (Perez et al. 2007). Gas dynamics within the gut are said to be physiologically modulated (Harder et al. 2004). The total volume of intestinal gas lies generally between 50 and 150 ml in human subjects, of which 63% is found in the colon (Perez et al. 2007). The quantity of gas produced is likely to vary with diet, notably the proportion of fermentable residue in vegetable matter (Becker et al. 2003).

The presence of small gas bubbles in watery organic particulate suspensions causes their physical properties to change (Briggs and Richardson 1996). The presence of fermentative bubbles is reported to increase the viscosity of faecal slurries with high ϕ (Gauglitz et al. 1995). The nature of the interaction between a dispersed gaseous phase, i.e. discrete bubbles, and the surrounding particulate elements of digesta varies with the ambient pressure, and with the physical properties of the neighbouring particles (Terrones and Gauglitz 2003). Bubbles in faecal slurries containing more elastic particles tend to coalesce with greater numbers of particles and assume a dendritic shape, while bubbles in slurries containing more rigid particle interact with fewer particles and retain a more rounded shape (Gauglitz et al. 1995).

Elements of the faecal water on the exterior surfaces of the particles are sufficiently mobile (Dural and Hines 1993) to enable films on adjacent particles to coalesce forming extensive networks linked by liquid bridges. Hence, in distal colonic digesta with a reduced liquid phase (high ϕ) and well-dispersed gaseous phase, the close approach of moist particles causes their liquid surface films to coalesce forming ‘funicular’ (Newitt and Conway-Jones 1958; York and Row 1994) bridges in which surface tension and capillary suction pressure act to hold the particles together (Iveson et al. 2001) (Fig. 6.4). Distraction of ‘bridged’ particles is resisted and cohesion is further augmented by the presence of any resistance to flow within the liquid bridge, i.e. the viscosity of the bridging liquid (Iveson et al. 2001).

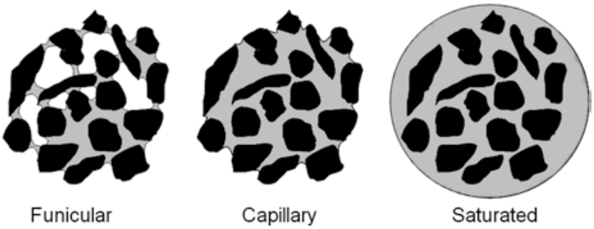


Fig. 6.4 Different states of saturation of liquid-bound particles. In the capillary regime, particles are held together predominantly by capillary suction pressure, while in the funicular state, surface tension also becomes significant

Reduction in the size of the particles within a cohesive mass increases the volume density of coalescence points, i.e. causes the 'particle coordination number' (Iveson and Page 2005; Rumpf 1962) and the magnitude of the volume-specific cohesive force to increase. Conversely, when the liquid content of a cohesive bolus is increased, the funicular liquid bridges expand and eventually merge to form a continuous 'capillary' network in which the liquid phase is continuous (Newitt and Conway-Jones 1958; York and Row 1994). In such a network, the contribution of surface tension is zero and cohesion is maintained by capillary suction pressure alone (Iveson et al. 2002). Further increases in saturation cause the liquid channels to widen and cohesive forces to drop to zero, whereupon the integrity of the bolus depends solely on viscous and direct frictional forces between adjacent particles (Iveson et al. 2001).

The presence of an extensive gaseous phase in more distal colonic digesta will generate cohesive forces that draw digesta particles closer together obstructing the flow of liquids through interparticle voids, i.e. reducing liquid permeability. This will aid aggregation of faecal particles but obstruct the expression of liquid (Helle and Paulapuro 2004; Rauch and Sangl 2000) and contained fine particulate material (Björnhag 1972, 1981a). Hence in the distal colon, the presence of bubbles and pore plugging by fine particulate material may act together to maintain the hydration of the matrix, i.e. faecal water content, and to reduce the mechanical resistance of the faecal mass during peristalsis. These effects may have a bearing on the genesis of pathophysiological conditions, such as diverticulosis, that are thought to result from high local intraluminal pressures (Bassotti et al. 2003). Further, the action of unbound mutagens in faecal water on the cells of the colonic mucosa may be limited by the presence of a dispersed gaseous phase.

The presence of an extensive gaseous phase in freshly swallowed food boluses may augment bolus strength and reduce the ease of permeation of boluses by gastric juices causing them to persist for longer in the gastric lumen (Fillery-Travis et al. 2002). This would prolong the action of any contained salivary amylase, and delay the bolus dispersal and transit to the small intestine.

6.4.4 Effect of Oil in Digesta

Oil droplets and emulsions within digesta may interact with the surfaces of particles, although high levels of mixing are required for this to occur. Thus indigestible mineral oils (Brunton 1990) and oily agents, such as indigestible sucrose esters of fatty acids (e.g. Olestra®), can soften the stool by generating oily funicular bridges, which are weaker than water-based bridges owing to their lower surface tension. In the latter case, this softening occurs in spite of a marked reduction in water content (McRorie et al. 2000).

Heterogeneous digesta particles, whether large or small, are likely to possess surfaces with differing degrees of hydrophobicity. Strongly hydrophilic regions of the surfaces of particles will tend to associate with the aqueous phase of digesta forming contact angles of < 90° at the solid–liquid interface, i.e. be readily wettable. Strongly hydrophobic regions of the surfaces of particles will associate less readily with the aqueous phase with contact angles of > 90° but will associate readily with oil droplets. If the surfaces of digesta particles are entirely hydrophilic they will be completely enveloped by the aqueous phase in an oil-in-water emulsion. If the surfaces of digesta particles are entirely hydrophobic and of sufficiently small size, they will be completely enveloped by the oil in a dispersed lipid droplet or in the continuous phase of a water-in-oil emulsion.

A particle that contains both hydrophilic and hydrophobic surfaces will orient across the oil–water interface of dispersed droplets in water-in-oil or oil-in-water emulsions and stabilise them (Finkle et al. 1923; Levine and Sanford 1985). Heterogeneous particles that are largely hydrophilic will align with the interface of coarse oil-in-water emulsions but will not prevent them from coalescing. Similarly, systems of heterogeneous particles that are largely hydrophobic will align with the interface of coarse water-in-oil emulsions but will not prevent them from coalescing (Binks and Lumsdon 2000). Conversely, systems of particles which are of intermediate hydrophobicity or hydrophilicity will align with the interface of fine

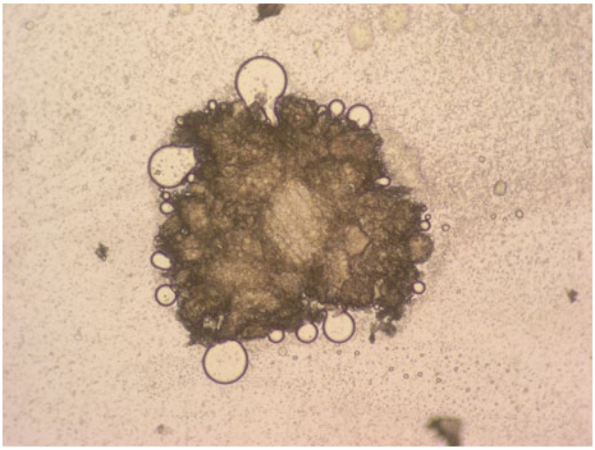


Fig. 6.5 Agglomerate of insoluble residues following in vitro digestion of pasta showing oil droplets that are partially expressed from the surface but remain bound to the hydrophobic surfaces of component particles

emulsions and prevent them from coalescing. These considerations indicate that the heterogeneous particulate residues of partially digested foods, such as occur in the stomach, may coat the oil–water interface of oil droplets and reduce their likelihood of coalescence (Fig. 6.5). In the case of the stomach, this may reduce their tendency to separate and float in the gastric fundus, and hence enhance their probability of transiting the pylorus.

6.5 Fractionation of Digesta

Work with selectively marked components of digesta shows that they can be retained for different times within particular segments of the gastrointestinal tract (Björnhag 1972). Relative retention of particles and the more rapid onflow of the liquid phase occur in the herbivore stomach (Mayer 1994), notably in ruminant (Hyden 1961; Balch and Campling 1965; Grovum and Williams 1973; Faichney 1975) and non-ruminant herbivore species, e.g. macropods (Hume 1999), horses (Argenzio et al. 1974) and llamas *(Lama guanacoe)* (Heller et al. 1986). Similar selective retention of particulate fractions has been reported in animals of other dietary habit and simpler stomach morphology, e.g. the hedgehog *(Erinaceus europaeus)*, raccoon *(Procyon lotor)* and dog (Stevens and Hume 1995). Again, relative retention of particles is reported to occur in other compartments of the gut, e.g. in the horse at the junction of the caecum with the colon (Argenzio et al. 1974), which have little obvious morphological specialisation (Stevens and Hume 1995).

In small-bodied animals whose overall digesta mean retention time (MRT) is necessarily short, some segments of the alimentary canal may operate to sequester the liquid phase or to selectively retain finer particles (Cork et al. 1999). Similar patterns of retention occur in larger-bodied animals that have specialised in the fermentative digestion of fibrous foods (Björnhag 1994; Karasov and Hume 1997).

To achieve differential retention of one of the two phases of digesta phases requires the exploitation of some difference in the properties of the phases, such as density or particle size. The separation and relative retention of the liquid and solid phases may also result in part from the ability of the solid particles to form a porous matrix through which the liquid can be expressed.

6.5.1 Buoyancy and Sedimentation of Particles

The importance of buoyancy in influencing the retention of particles within the stomach and prolonging their solution and digestion is exemplified by pharmaceutical experimentation with floating drug delivery systems (Singh and Kim 2000). Thus when taken after a meal, floating capsules are retained in the stomach for longer periods than non-floating systems regardless of the capsule size, provided the subject is maintained in the upright position.

A number of food items contain air-filled spaces or significant accumulations of fat that may remain in situ after mastication and deglutition, and contribute to the buoyancy of solid digesta particles formed from them. Conversely, digesta particles containing a higher proportion of lignin may be more dense and likely to sink (Lirette et al. 1990). Significant aggregates of fat may also liquefy as they warm to body temperature and coalesce to float as a fatty layer on the watery liquid phase (Boulby et al. 1997).

The relative buoyancy of a particle or fat droplet may influence its rate of passage through a particular segment of the digestive tract (Amidon et al. 1991), but this is only likely in segments of the gut where flow rates are low, digesta is sufficiently fluid, and the segment is of a sufficient volume to allow separation, notably the stomach (Boulby et al. 1997) and the proximal sacculated colon (Brown et al. 1995). In an organ of sacculate configuration, such as the stomach, the effect of buoyancy may also be dependent on posture, e.g. the transit of floating fat through the pylorus varies with body position (Boulby et al. 1997).

Radio-labelling studies show that fats that are held in situ within cellular matrices empty from the stomach with the solid phase of the meal (Edelbroek et al. 1992) but that fat that has separated from the solid component of the meal and is free floating within the lumen of the stomach empties more slowly than does the watery phase of gastric digesta (Cunningham et al. 1991; Jian et al. 1982). The retention of emulsified fat depends upon the stability of the emulsion within the stomach and the position of the subject. Acid-stable emulsions are reported to exit more slowly than those which break (Marciani et al. 2009), provided the subject is lying down in a position in which floating globules of fat liberated from the broken emulsion are prevented from entering the pylorus.

6.5.2 Filtration and Expression

The difficulties of mixing semisolid digesta make alternative less energy-intensive strategies of digestion more viable. Hence sections of the gut that contain semisolid digesta may operate to selectively retain and digest finer particles in an operation that is broadly analogous to that of a commercial biomass feedback fermenter (Stanbury et al. 1995).

Fine particles and liquid are selectively retained in the colon of the rabbit. The mechanical basis of the colonic separating mechanism (CSM) (Björnhag 1981b; Sperber et al. 1983) has been variously explained by terms such as ‘pressing out’ (Sakaguchi 2003) or ‘squeezing’ (Hörnicke and Björnhag 1980). It has been suggested that this results from intestinal motility patterns such as the ‘rolling’ movements of colonic haustra (Ehrlein et al. 1982). The process that appears to be instigated by the action of such contractions is one of filtration or expression (Lentle et al. 2009) of the liquid phase through a mat formed from larger particulate elements of the solid phase. The expression of the fluid from the solid phase requires a suitable intestinal morphology as well as a solid phase with properties that are suitable for the formation of a mat or porous matrix of particles.

6.5.2.1 The Mechanism of Cake Formation

The process of filtration of a particulate suspension with low solid volume fraction *(ϕ)* can be considered to consist of two stages (Fig. 6.6). In the initial 'cake formation' stage, where particulate material is retained at a constriction point and accumulates to form a cake, while the liquid phase continues to flow onwards. In the following 'cake compression' stage, the cake becomes progressively compacted, and contained liquid is expressed from the diminishing pore spaces between the particles (Wakeman and Tarleton 1999).

During the initial stage, the ϕ of digesta at the filtration point must rise above the critical solid volume fraction *(ϕ_c)*, which is the concentration at which the solid network is able to become contiguous and form a three-dimensional matrix (Landman and White 1997). The formation of a cake of particles will generate resistance to flow causing a pressure differential to develop across the cake. The compressive yield stress *($\sigma_y(\phi)$)* of a three-dimensional matrix of particles, increases in direct relation to ϕ in a three-dimensional particle matrix (Buscall and White 1987). Hence any cake that is unable to resist the load resulting from the pressure differential

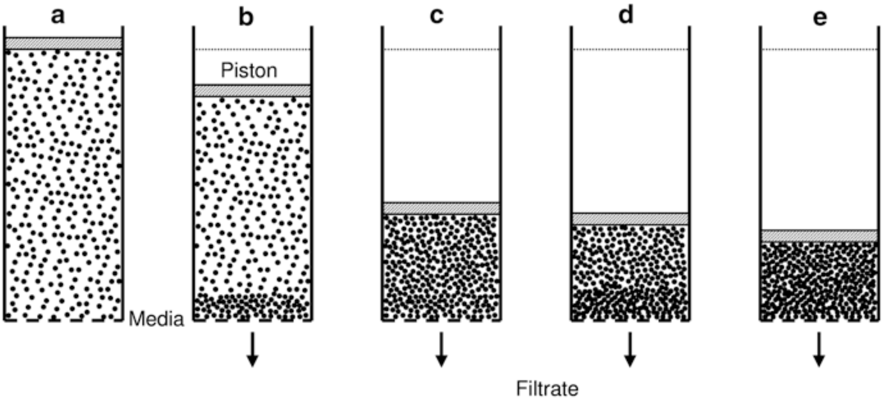


Fig. 6.6 Showing the mechanism of filtration and expression of a dilute suspension with uniformly distributed particles (**a**). During the first stage (**b**), filter cake or matrix formation commences at the point of constriction (lower border of cylinder) and propagates inwards, eventually (**c**) filling the available volume. During the subsequent expression stage (**d**), the matrix becomes further compressed at the constriction point, the compression again propagating inwards until a steady state (**e**) is reached when the applied force is exactly balanced by the resistance of a uniformly compressed matrix

across it will collapse causing ϕ to increase locally to the point where the yield stress balances the applied stress. Provided the cake is able to withstand the pressure differential, it will build back from the constriction point toward the region of higher pressure.

If the ϕ of the original suspension is higher than ϕ_c, then the extensive particle interactions necessary for cake formation will have already occurred, and filtration can only occur by cake compression. The onset of the compression stage, with concomitant expression of the liquid phase, will occur when the applied load exceeds the compressive yield stress of the existing particulate matrix. The portion of the existing matrix that is immediately adjacent to the constriction point will collapse first, causing the liquid phase to be expressed and ϕ to increase locally to the point where the yield stress balances the applied stress (Buscall and White 1987). This local increase of ϕ will progressively propagate in a retrograde manner through the cake establishing a more compact matrix throughout the sample that has sufficient strength to resist the new load (Landman and White 1997). Further increases in the pressure differential across the denser cake will generate further cycles of retrogradely propagating matrix compression.

The rate at which liquid is expressed from the matrix will decrease progressively over time as the mean pore size is reduced and the mean distance travelled by liquid through the matrix increases (Landman and White 1997). Generally, the rate of expression of liquid decays exponentially when a constant pressure differential is applied (Wakeman and Tarleton 1999). Successive increments in pressure lead to repeated expression cycles (Kugge et al. 2005) with progressive reduction in the amount by which the volume of the matrix and the volume of liquid expressed

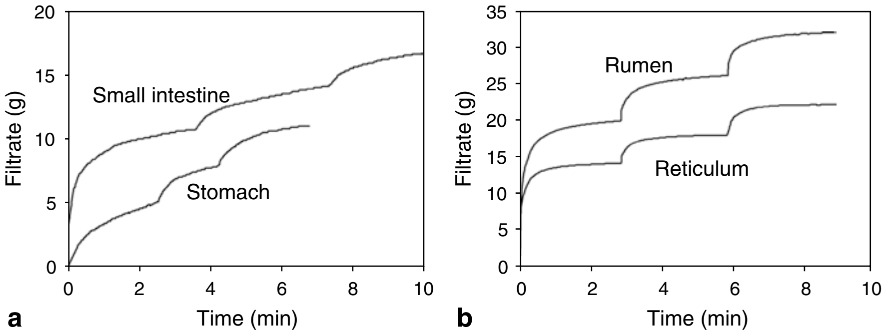


Fig. 6.7 Filtration curves of digesta from the stomach and small intestine of the brushtail possum (**a**), and the rumen mat and reticulum of sheep (**b**). The pressure was increased in steps to 2.5 kPa, 5 kPa and 10 kPa and the volume of fluid expressed from a 75-g sample was measured continuously. Note the greater initial volume of filtrate and the longer time taken for the volume to equilibrate with new pressure in digesta from the distal small intestine compared to that from the stomach of the brushtail possum, and the greater volume expressed from ruminal than from reticular digesta (but similar time to come to equilibrium) Digesta from the caecum and proximal colon of the possum failed to yield any filtrate on application of these pressures. (Reproduced from Lentle et al. 2009)

are reduced, until a point is reached where the pressure required to induce further matrix compression is above the physiological range and further expression of the liquid phase no longer practicable.

Measurements of the filtration rate of the liquid phase from animal digesta (Fig. 6.7) show that it behaves similarly to other suspensions of biological materials (Schwartzberg 1997). Further, that the extent of matrix collapse and the proportion of liquid expressed from digesta on application of pressures in the physiological range are less in digesta from the distal gut than that from the proximal gut. No expression data are available for human colonic digesta, but there are grounds to suspect that expression of the liquid phase and/or separation of fine particles may occur, if only at a local level. No liquid is expressed from the digesta from the unsacculated caecum and proximal colon of the brushtail possum, an arboreal folivore, on application of physiological pressures (Lentle et al. 2009), and it is noteworthy that there is no selective particle retention in this species (Foley and Hume 1987). However, species such as the rabbit, which exhibit a similar sacculated structure to that of the human proximal colon, are known to selectively retain finer particles (Björnhag 1981b). Further, studies of the passage of markers through the right (ascending) human colon show selective retention of finer particles (Davis et al. 1984a, b; Hardy et al. 1985; Proano et al. 1990).

6.5.2.2 The Nature of the Constriction Point

Industrial and laboratory filtration processes use a filter media, e.g. cloth, paper or gauze, to provide an array of constriction points in the flow-path of the suspension. With the possible exception of the ruminant omasum, no analogous structure is apparent in the mammalian gastrointestinal tract. However, it is well known that the pore size of industrial media can be considerably larger than the sizes of particles that they are able to retain. This discrepancy results from the formation of ‘bridges’ of interacting particles that span the pores, effectively decreasing their diameter (Cheremisinoff 1998). Similarly, in the gastrointestinal tract, filtration and expression can be achieved at a single aperture or slit that is narrow enough to be bridged by digesta particles. Such openings may be more reliable in an evolutionary sense than a filter structure that could become permanently blocked, as their diameter may be adjusted by associated musculature. A number of specific sites for filtration and expression associated with selective retention of fine nutrient-rich digesta particles have been identified in vertebrate species, such as the guinea pig (Takahashi and Sakaguchi 2006), lemming (Sperber et al. 1983), and various species of bird (Fenna and Boag 1974), but as yet none have been identified in the human hind gut.

The fact that bridging of particles across a constriction point is promoted at higher concentrations of particulate matter (Cheremisinoff 1998) may facilitate filtration in more distal regions of the gut. The ease of particle bridging is also influenced by the size of the particle relative to that of the pore, and by the same factors that influence matrix formation, namely the forces between particles such as adhesion and friction, and the tendency of particles with a high aspect ratio to become entangled.

6.5.2.3 The Pressure Differential

While the selective retention of solids and the onflow of liquids and fine particles can result from matrix compression, it is important to note that such a process may be mechanically disadvantageous. Both the rate of cake formation and the rate of expression of the liquid phase, i.e. the flow of liquid through the porous structure of the matrix, are dependent on the pressure differential across the point of constriction. To a first approximation, the permeation rate will be proportional to the square root of the pressure difference applied. Hence large increments in the applied pressure differential are required to achieve small increases in the expression rate of liquid and fine nutrient-rich particles from the digesta matrix. This is evident in the chicken, where a pressure differential that is sufficient to express liquid and fine nutrient-dense particles from avian colonic digesta into the paired caeca requires concerted antiperistaltic colonic contractions and peristaltic contractions in the ileum (Fenna and Boag 1974), possibly with further augmentation by negative pressure generated in the narrow thick-walled proximal portion of the caecum (Janssen et al. 2009). High intraluminal pressures may be pathophysiological, e.g. the development of high pressure in the human colonic lumen has been linked to diverticulosis (Arfwidsson et al. 1964; Painter and Truelove 1964; Painter et al. 1965; Trotman and Misiewicz 1988). Consequently, dietary constituents that increase the permeability of the particulate matrix of colonic digesta may beneficially reduce colonic pressure differential. Further, they may increase the mean diameter of the colon, and hence, lower the luminal pressure generated by colonic peristalsis (Painter and Burkitt 1971, 1975).

Particles of mixed sizes, i.e. polydisperse, can pack together on compression at higher densities than do particles of a more uniform size (Dodds 1980). The smaller particles can occupy and narrow the spaces between larger particles causing frictional forces on the contained liquid phase to increase so that it is less easily expressed (Abe et al. 1979; MacDonald et al. 1991). Thus there may be an advantage in creating a preponderance of larger particles and reducing the relative proportion of fine particles in colonic digesta, for example, by consumption of coarse dietary fibre.

The flexibility of particles may also influence the ease with which the liquid phase is expressed. More flexible fibres may be more readily forced together to 'seal' off the flow (Steadman and Luner 1985), while stiffer fibres tend to maintain pore size and the continuing expression of the liquid phase (Britt 1981). Where fibres are flexible and friction between them is low, groups of them may approximate to form contiguous sheets causing 'sealing' to occur over large areas. It is doubtful whether such sheet formation occurs in hindgut digesta as MRI scans conducted in our laboratory on faeces from various herbivores do not show faecal fibres to be aligned in planes.

The liquid phase is less readily expressed from matrices that are composed of particles in which cell wall fibres have become delaminated than from those composed of more 'block-shaped' particles with lower specific surface area (Hubbe 2002; Krogerus et al. 2002). The ease with which the liquid phase is expressed from matrices composed of delaminated cellulose fibres is increased following their treatment with cellulase, an effect that is thought to result from the reduction of liquid phase friction by removal of projecting fibrillar material and the 'polishing' of their surfaces (Gong and Bi 2005). However, those surface properties of fibres which reduce the ease with which fibres can slide past each other may act conversely to hinder fibre compaction and to increase the ease with which the liquid phase is expressed by maintaining larger pore spaces for longer (Hubbe 2001; Sampson and Kropholler 1995).

6.5.2.4 The Effects of Fine Particles

Any fine particulate material that is not large enough to be trapped within the percolating network of larger particles may be expelled with the liquid phase, as was observed in the filtration of digesta in Fig. 6.7. Indeed, such expression has been postulated to form the basis for the colonic separating mechanism (CSM), i.e. the selective retention of fine nutrient-rich particles in the hindgut of rabbits (Björnhag 1981b). This hypothesis is supported by evidence that fine particles are able to migrate within a matrix of cellulose fibres (Hubbe and Heitmann 2007) and that the majority do not become attached to the fibrous components of the matrix during expression of the liquid phase (Britt 1981). If the concentration of larger particles is sufficient to form an entangled network, i.e. a mat, some separation of the fine particle content may be achieved simply by the application of a pressure differential across the mat.

The liquid phase is expressed more readily from matrices of coarse than from those of fine particles (Przybysz and Szwarcsztajn 1973). This is thought to result principally from the higher surface area per unit mass, leading to higher frictional resistance to liquid flow (Hubbe and Heitmann 2007). This effect may account for the observation that the CSM does not appear to operate when rabbits are fed more finely ground food (Björnhag 1994).

The flow of liquid from a matrix may also be reduced by 'pore plugging'. This occurs when the migration of fine particles through a particulate matrix is halted in areas where pore diameters have become too small to allow their transit. Pore plugging by bacteria could bring about a longstanding reduction in the ease with which the liquid phase is expressed from hindgut digesta. Pore plugging generally occurs during the propagation of collapse across a matrix. Hence, the collapse of a matrix adjacent to a constriction point, with local reduction in pore size, may form a 'choke point' (Hubbe and Heitmann 2007) that arrests the passage of fine particles that have successfully traversed the proximal portion of the matrix which has not yet collapsed (Wildfong et al. 2000). The time constants for the expression of liquid from samples of digesta taken from the stomach and distal small intestine of the brushtail possum, and from the rumen and reticulum of the sheep are similar after repeated increments in pressure (Fig. 6.7). This indicates that pore plugging of digesta matrices is not significant at these sites (Lentle et al. 2009).

The ease of migration of fine particles through the matrix is increased, and the extent of pore plugging and sealing is reduced, by agitation (Britt 1981) and other processes that induce oscillatory flow in the liquid phase (Mitchell and Johnston 2000). Further, the spacing and duration of such oscillation influence the efficiency of liquid phase expression (Räisänen 1996). Hence, the motility patterns described as fast phasic contractions and colonic rippling (Lentle et al. 2008) (see Chap. 9) may not only aid expression of the liquid phase but also prevent or reduce pore plugging.

6.5.2.5 The Elasticity of the Matrix

The reduction of the volume of a contiguous network of particles during contractile activity will cause some of the liquid phase contained within the network to be expressed from it. If the contiguous network is perfectly elastic and remains sufficiently permeable after compression, this liquid may be entirely reabsorbed into the network during its subsequent re-expansion after cessation of the contraction (Lentle et al. 2005). Conversely, in situations where the applied forces are sufficient to cause elements of the contiguous network to undergo plastic deformation or to fail, contractile activity may bring about a permanent reduction in the volumes of the void spaces between these elements, i.e. compaction, reducing subsequent permeability and increasing ϕ. Such changes may be more likely to occur in digesta that have entered the hindgut, and undergone repeated and more powerful peristalsis (Lentle et al. 2005). Hence, the strength, elasticity and volume fraction of the solid phase may influence the extent of liquid expression from and its subsequent re-absorption into a particulate aggregate.

Recent work (Lentle et al. 2006) has examined this effect in herbivore colonic digesta via creep rheometry, a technique that assesses the time-dependent elastic behaviour of contiguous networks of particles in digesta during compression and recovery. These studies show that such structures undergo significant re-expansion between successive peristalses, but that recovery is generally incomplete. The creep recovery profiles of digesta from the proximal and distal colon did not differ, indicating that there was little degradation of elastic properties of the solid phase of distal colonic digesta despite a significant increase in dry matter content (Lentle et al. 2005).

If the volume of a contiguous particulate network is progressively reduced by repeated peristalsis during the distal progression of a bolus though the intestinal tract, then some of the displaced liquid phase may be driven distally in advance of the bolus. This may account for the significantly faster transit of liquid phase with respect to the solid phase in the human terminal ileum (Hammer et al. 1993; Hebden et al. 1998).

6.6 Effects of Biofilms on Digesta

In the colon at least, the surfaces of digesta particles may be coated with biofilms composed of enteral microbial consortia. Given that microbial biofilms may only be eroded at shear rates that are relatively high (Ymele-Leki and Ross 2007) in respect of the contractile physiology of the colon (see Chap. 9), it is likely that they may influence the physical properties of digesta by increasing the size of particles, and modifying their surface characteristics and viscoelastic properties. A body of work has described the rheology of biofilms in conduits (Stoodley et al. 1999), but the morphology of such colonies is filamentous and likely to differ from those on digesta particles, given that biofilm structure is known to be influenced by local flow conditions (Stoodley et al. 2000). To date, we can find no work that has described the rheology of biofilms on particulate digesta or examined its contribution to the rheology of whole colonic digesta. However, a number of reports indicate that biofilms grown under turbulent flow conditions in pipes may behave either as elastic solids, viscoelastic solids or as viscoelastic liquids according to the rate at which shear is applied (Stoodley et al. 1999), but these biofilms are inhomogeneous on a macrorheological (Stoodley et al. 1999) and microrheological scale (Rogers et al. 2008).

References

Abe E, Hirosue H, Yokota A (1979) Pressure drop through a packed bed of binary mixture. J Chem Eng Japan 12:302-306

Amerah AM, Lentle RG, Ravindran V (2007) Influence of feed form on gizzard morphology and particle size spectra of duodenal digesta in broiler chickens. J Poultry Sci 44:175-181

Amidon GL, Debrincat GA, Najib N (1991) Effects of gravity on gastric-emptying, intestinal transit, and drug absorption. J Clin Pharmacol 31:968-973

Arfwidsson S, Knock N, Lehmann L, Winberg T (1964) Pathogenesis of multiple diverticula of the sogmoid colon in diverticular disease. Acta Chir Scand Suppl 63

Argenzio RA, Lowe JE, Pickard DW, Stevens CE (1974) Digesta passage and water exchange in the equine large intestine. Am J Physiol 226:1035-1042

Balch CC, Campling RC (1965) Rate of Passage of Digesta Through the Ruminant Digestive Tract. In: Dougherty RW (ed) Physiology of Digestion in the Ruminant. Butterworths, Washington, pp 108-123

Barnes HA (1999) The yield stress - a review or 'παντα ρει' - everything flows? J Non-Newtonian Fluid Mech 81:133-178

Barnes HA, Hutton JF, Walters K (1989) An introduction to rheology. Elsevier, Amsterdam

Bassotti G, Chistolini F, Morelli A (2003) Pathophysiological aspects of diverticular disease of colon and role of large bowel motility. World J Gastroenterol 9:2140-2142

Becker PM, Van Gelder AH, Van Wikselaar PG, Jongbloed AW, Cone JW (2003) Carbon balances for in vitro digestion and fermentation of potential roughages for pregnant sows. Anim Feed Sci Tech 110:159-174

Bennington CPJ, Kerekes RJ, Grace JR (1990) The yield stress of fibre suspensions. Can J Chem Eng 68:748–757

Binder HJ (1983) Absorption and secretion of water and electrolytes by small and large intestine. In: Sleisinger O, Fordtran O (eds) Gastrointestinal disease. Saunders, Philadelphia, pp 812-829

Binks BP, Lumsdon SO (2000) Influence of particle wettability on the type and stability of surfactant-free emulsions. Langmuir 16:8622-8631

Björnhag G (1972) Separation and delay of contents in the rabbit colon. Swed J Agr Res 2:125–136

Björnhag G (1981a) The retrograde transport of fluid in the proximal colon of rabbits. Swed J Agr Res 6:63–69

Björnhag G (1981b) Separation and retrograde transport in the large intestine of herbivores. Livestock Prod Sci 8:351-360

Björnhag G (1994) Adaptations in the large intestine allowing small animals to eat fibrous foods. In: Chivers DJ, Langer P (eds) The digestive system in mammals; food form and function. University Press, Cambridge pp 287-309

Borgstrom S, Arborelius M (1975) Influence of a fatty acid on duodenal motility. Scand J Gastroenterol 10:599-601

Boulby P, Gowland P, Adams V, Spiller RC (1997) Use of echo planar imaging to demonstrate the effect of posture on the intragastric distribution and emptying of an oil/water meal. Neurogastroenterol Mot 9:41-47

Briggs KB, Richardson MD (1996) Variability in in situ shear strength of gassy muds. Geo-Marine Letters 16:189-195

Britt KW (1981) Observations on water removal during the papermaking process. Tappi J 64:55-56

Brown BP, Schrier JE, Berbaum KS, Shirazi SS, Schulze-Delrieu K (1995) Haustral septations increase axial and radial distribution of luminal contents in glass models of the colon. Am J Physiol 269:G706-709

Brunton LL (1990) Agents affecting gastrointestinal water flux and motility, digestants, and bile acids. In: Gilman AG, Rall TW, Nies AS, Taylor P (eds) The pharmacological basis of therapeutics. Pergamon, Elmsford, pp 914-932

Buscall R, White LR (1987) The consolidation of concentrated suspensions. Part 1. The theory of sedimentation. J Chem Soc Faraday Trans 1 83:873-891

Buxton D, Redfearn D (1997) Plant limitations to fiber digestion and utilization. J Nutr 127:814S

Cameron-Smith D, Collier GR, O’Dea K (1994) Effect of soluble dietary fibre on the viscosity of gastrointestinal contents and the acute glycaemic response in the rat. Br J Nutr 71:563-571

Cheremisinoff NP (1998) Liquid filtration. Butterworth-Heinemann, Boston

Cork SJ, Hume ID, Faichney GJ (1999) Digestive strategies of nonruminant herbivores: the role of the hindgut. In: Jung H-JG, Fahey GC (eds) Nutritional Ecology of Herbivores. Am Soc Animal Sci, Savoy, pp 210-260

Coussot P, Ancey C (1999) Rheophysical classification of concentrated suspensions and granular pastes. Phys Rev E 59:4445-4457

Crank J (1975) The mathematics of diffusion. Oxford University Press, Oxford

Cunningham KM, Baker RJ, Horowitz M, Maddox AF, Edelbroek MAL, Chatterton BE (1991) Use of technetium-99m(V)thiocyanate to measure gastric emptying of fat. J Nucl Med 32:878-881

da Silva JAL, Rao MA (1999) Rheological behavior of food gel systems. In: Rao MA (ed) Rheology of fluid and semisolid foods. Aspen, Gaithersburg, pp 319–368

Davis SS, Hardy JG, Taylor MJ, Stockwell A, Whalley DR, Wilson CG (1984a) The *in-vivo* evaluation of an osmotic device (Osmet) using gamma scintigraphy. J Pharm Pharmacol 36:740-742

Davis SS, Hardy JG, Taylor MJ, Whalley DR, Wilson CG (1984b) A comparative study of the gastrointestinal transit of a pellet and tablet formulation. Int J Pharm 21:167-177

Defilippi C, Gomez E (1995) Effect of casein and casein hydrolysate on small bowel motility and d-xylose absorption in dogs. Neurogastroenterol Mot 7:229-234

Dikeman CL, Barry KA, Murphy MR, Fahey JGC (2007) Diet and measurement techniques affect small intestinal digesta viscosity among dogs. Nut Res 27:56-65

Dikeman CL, Fahey GC (2006) Viscosity as related to dietary fiber: A review. Crit Rev Food Sci Nutr 46:649-663

Dodds JA (1980) The porosity and contact points in multicomponent random sphere packings calculated by a simple statistical geometric model. J Colloid Interface Sci 77:317-327

Doi M, Edwards SF (1986) The theory of polymer dynamics. Oxford University Press, Oxford

Dural NH, Hines AL (1993) Adsorption of water on cereal-bread type dietary fibers. J Food Eng 20:17-43

Eastwood MA (2007) Vegetable fibre: Its physical properties. Proc Nutr Soc 32:137-143

Eastwood MA, Hamilton D (1968) Studies on the adsorption of bile salts to non-absorbed components of diet. Biochim Biophys Acta 152:165-173

Eastwood MA, Robertson JA, Brydon WG, MacDonald D (1983) Measurement of water-holding properties of fibre and their faecal bulking ability in man. Br J Nutr 50:539-547

Edelbroek M, Horowitz M, Maddox A, Bellen J (1992) Gastric emptying and intragastric distribution of oil in the presence of a liquid or a solid meal. J Nucl Med 33:1283-1290

Ehrlein HJ, Reich H, Schwinger M (1982) Physiological significance of the contractions of the rabbit proximal colon. Q J Exp Psychol 67:407-417

Faichney GJ (1975) The use of markers to partition digestion within the gastro-intestinal tract of ruminants. In: McDonald IW, Warner ACI (eds) Digestion and metabolism in the ruminant. University of New England, Armidale, pp 277-291

Fenna L, Boag DA (1974) Filling and emptying of the galliform caecum. Can J Zool 52:537-540

Ferry JD (1980) Viscoelastic properties of polymers. Wiley, New York

Fillery-Travis A, Marciani L, Gowland PA, Spiller RC (2002) The role of polysaccharides in the gastric processing of foods. In: Williams PA, Phillips GO (eds) Gums and stabilisers for the food industry 11. RSC, Cambridge, pp 342–348

Finkle P, Draper HD, Hildebrand JH (1923) The theory of emulsification. J Am Chem Soc 45:2780-2788

Foley WJ, Hume ID (1987) Passage of digesta markers in two species of arboreal folivorous marsupials - the greater glider (*petauroides volans*) and the brushtail possum (*trichosurus vulpecula*). Physiol Zool 60:103-113

France J, Thornley JHM, Siddons RC, Dhanoa MS (1993) On incorporating diffusion and viscosity concepts into compartmental models for analysing faecal marker excretion patterns in ruminants. Br J Nutr 70:369-378

Gauglitz PA, Rassat SD, Powell MR, Shah RR, Mahoney LA (1995) Gas bubble retention and its effects on waste properties: Retention mechanisms, viscosity, and tensile and shear strength. Report PNL-10740. Pacific Northwest Laboratory, Richland

Gong MR, Bi SL (2005) Mechanism of drainage improvement of bleached wheat pulp by the functioning of cellulase. China Pulp Paper 24:1-4

Grovum WL, Williams VJ (1973) Rate of passage of digesta in sheep. 1. The effect of level of food intake on marker retention times along the small intestine and on apparent water absorption in the small and large intestines. Br J Nutr 29:13-21

Gwynne RM, Thomas EA, Goh SM, Sjovall H, Bornstein JC (2004) Segmentation induced by intraluminal fatty acid in isolated guinea-pig duodenum and jejunum. J Physiol 556:557-569

Hammer J, Camilleri M, Phillips SF, Aggarwal A, Haddad AM (1993) Does the ileocolonic junction differentiate between solids and liquids? Gut 34:222-226

Harder H, Serra J, Azpiroz F, Malagelada JR (2004) Reflex control of intestinal gas dynamics and tolerance in humans. Am J Physiol 286:G89-94

Hardy JG, Wilson CG, Wood E (1985) Drug delivery to the proximal colon. J Pharm Pharmacol 37:874-877

Hebden JM, Blackshaw PE, Perkins AC, D'Amato M, Spiller RC (1998) Small bowel transit of a bran meal residue in humans: Sieving of solids from liquids and response to feeding. Gut 42:685-689

Hecker JF, Grovum WL (1975) Rates of passage of digesta and water absorption along the large intestines of sheep, cows and pigs. Aust J Biol Sci 28:161-167

Helle TM, Paulapuro H (2004) Effect of precipitated gas bubbles in papermaking. APPITA J 57:444-447

Heller R, Cercasov V, Engelhardt WV (1986) Retention of fluid and particles in the digestive tract of the llama (*Lama guanacoe F. Glama*). Comp Biochem Physiol 83A:687-691

Heller SN, Hackler LR, Rivers JM, Van Soest PJ, Roe DA, Lewis BA, Robertson J (1980) Dietary fiber: The effect of particle size of wheat bran on colonic function in young adult men. Am J Clin Nutr 33:1734-1744

Hetland H, Choct M, Svihus B (2004) Role of insoluble non-starch polysaccharides in poultry nutrition. Worlds Poult Sci J 60:415-422

Hetland H, Svihus B (2001) Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. Br Poult Sci 42:354-361

Hörnicke H, Björnhag G (1980) Coprophagy and related strategies for digesta utilization. In: Ruckebusch Y, Thivend P (eds) Digestive Physiology and Metabolism in Ruminants. MTP Press, Lancaster, pp 707–730

Hubbe MA (2001) Reversibility of polymer-induced fiber flocculation by shear. 2. Multi-component chemical treatments. Nordic Pulp Paper Res J 16:369-375

Hubbe MA (2002) Fines management for increased paper machine productivity. Proceedings of Scientific and Technical Advances in Wet End Chemistry. Pira International, Vienna

Hubbe MA, Heitmann JA (2007) Review of factors affecting the release of water from cellulosic fibers during paper manufacture. BioResources 2:500-533

Huge A, Weber E, Ehrlein HJ (1995) Effects of enteral feedback inhibition on motility, luminal flow, and absorption of nutrients in proximal gut of minipigs. Dig Dis Sci 40:1024-1034

Hume ID (1999) Marsupial Nutrition. Cambridge University Press, Cambridge

Hyden S (1961) The use of reference substances and the measurement of flow in the alimentary tract. In: Lewis D (ed) Digestive physiology and nutrition of the ruminant. Butterworths, London, pp 35-47

Itoh T, Higuchi T, Gardner CR, Caldwell L (1986) Effect of particle size and food on gastric residence time of non-disintegrating solids in beagle dogs. J Pharm Pharmacol 38:801-806

Iveson SM, Beathe JA, Page NW (2002) The dynamic strength of partially saturated powder compacts: The effect of liquid properties. Powder Technol 127:149-161

Iveson SM, Litster JD, Hapgood K, Ennis BJ (2001) Nucleation, growth and breakage phenomena in agitated wet granulation processes: A review. Powder Technol 117:3-39

Iveson SM, Page NW (2005) Dynamic strength of liquid-bound granular materials: The effect of particle size and shape. Powder Technol 152:79-89

Janssen PWM, Lentle RG, Asvarujanon P, Chambers P, Stafford KJ, Hemar Y (2007) Characterisation of flow and mixing regimes within the ileum of the brushtail possum using residence time distribution analysis with simultaneous spatio-temporal mapping. J Physiol 582:1239–1248

Janssen PWM, Lentle RG, Hulls C, Ravindran V, Amerah AM (2009) Spatiotemporal mapping of the motility of the isolated chicken caecum. J Comp Physiol B179:593-604

Jian R, Vigneron N, Najean Y, Jacques Bernier J (1982) Gastric emptying and intragastric distribution of lipids in man. Dig Dis Sci 27:705-711

Karasov WH, Hume ID (1997) Vertebrate gastrointestinal system. In: Dantzler WH (ed) Handbook of physiology, section 13: Comparative physiology, vol 1. Oxford University Press, Oxford, pp 409-480

Krieger IM, Dougherty TJ (1959) A mechanism for non-Newtonian flow in suspensions of rigid spheres. J Rheol 3:137-152

Krogerus B, Fagerholm K, Tiikkaja E (2002) Fines from different pulps compared by image analysis. Nordic Pulp Paper Res J 17:440-444

Kugge C, Bellander H, Daicic J (2005) Pressure filtration of cellulose fibres. J Pulp & Paper Sci 31:95-100

Landman KA, White LR (1997) Predicting filtration time and maximizing throughput in a pressure filter. AIChE J 43:3147-3160

Lentle RG, Hemar Y, Hall CE (2006) Viscoelastic behaviour aids extrusion from and reabsorption of the liquid phase into the digesta plug: Creep rheometry of hindgut digesta in the common brushtail possum *trichosurus vulpecula*. J Comp Physiol B176:469-475

Lentle RG, Hemar Y, Hall CE, Stafford KJ (2005) Periodic fluid extrusion and models of digesta mixing in the intestine of a herbivore, the common brushtail possum (*trichosurus vulpecula*). J Comp Physiol B175:337-347

Lentle RG, Janssen PWM, Asvarujanon P, Chambers P, Stafford KJ, Hemar Y (2007) High definition mapping of circular and longitudinal motility in the terminal ileum of the brushtail possum *trichosurus vulpecula* with watery and viscous perfusates. J Comp Physiol B177:543-556

Lentle RG, Janssen PWM, Asvarujanon P, Chambers P, Stafford KJ, Hemar Y (2008) High definition spatiotemporal mapping of contractile activity in the isolated proximal colon of the rabbit. J Comp Physiol B178:257-268

Lentle RG, Janssen PWM, Hume ID (2009) The roles of filtration and expression in the processing of digesta with high solid phase content. Comp Biochem Physiol A 154:1-9

Lentle RG, Stafford KJ, Kennedy MS, Haslett SJ (2002) Rheological properties of digesta suggest little radial or axial mixing in the forestomach of the tammar (*macropus eugenii*) and the parma (*macropus parma*) wallaby. Physiol Biochem Zool 75:572-582

Levenspiel O (1999) Chemical reaction engineering. Wiley, New York

Levine S, Sanford E (1985) Stabilization of emulsion droplets by fine powders. Can J Chem Eng 62:256–268

Lirette A, Milligan L, Cyr N, Elofson R (1990) Buoyancy separation of particles of forage, feces, and ruminal contents and nuclear magnetic resonance examination. Can J Anim Sci 70:1099–1108

Lucas P (2004) Dental functional morphology. Cambridge University Press, Cambridge

MacDonald MJ, Chu C, Guilloit PP, Ng KM (1991) A generalized blake-kozeny equation for multisized spherical particles. AIChE J 37:1583-1588

Mandalari G, Faulks RM, Rich GT, Lo Turco V, Picout DR, Lo Curto RB, Bisignano G, Dugo P, Dugo G, Waldron KW (2008) Release of protein, lipid, and vitamin E from almond seeds during digestion. J Agric Food Chem 56:3409-3416

Marciani L, Faulks R, Wickham MSJ, Bush D, Pick B, Wright J, Cox EF, Fillery-Travis A, Gowland PA, Spiller RC (2009) Effect of intragastric acid stability of fat emulsions on gastric emptying, plasma lipid profile and postprandial satiety. Br J Nutr 101:919-928

Marlett JA, Kajs TM, Fischer MH (2000) An unfermented gel component of psyllium seed husk promotes laxation as a lubricant in humans. Am J Clin Nutr 72:784-789

Martinez-Ruvalcaba A, Chornet E, Rodrigue D (2007) Viscoelastic properties of dispersed chitosan/xanthan hydrogels. Carbohydr Polym 67:586-595

Mayer EA (1994) The physiology of gastric storage and emptying. In: Johnson LR (ed) Physiology of the Gastrointestinal Tract. Raven, New York, pp 929-976

McClements D, Decker E, Park Y, Weiss J (2008) Designing food structure to control stability, digestion, release and absorption of lipophilic food components. Food Biophysics 3:219-228

McDonald DE, Pethick DW, Mullan BP, Hampson DJ (2001) Increasing viscosity of the intestinal contents alters small intestinal structure and intestinal growth, and stimulates proliferation of enterotoxigenic *Escherichia coli* in newly-weaned pigs. Br J Nutr 86:487-498

McRorie J, Brown S, Cooper R, Givaruangsawat S, Scruggs D, Boring G (2000) Effects of dietary fibre and olestra on regional apparent viscosity and water content of digesta residue in porcine large intestine. Aliment Pharmacol Ther 14:471–477

McRorie J, Pepple S, Rudolph C (1998) Effects of fiber laxatives and calcium docusate on regional water content and viscosity of digesta in the large intestine of the pig. Dig Dis Sci 43:738-745

Mewis J, Macosko CW (1993) Suspension rheology. In: Macosko CW (ed) Rheology: Principles, measurements, and applications. VCH, New York, pp 425–474

Meyer JH, Ohashi H, Jehn D, Thomson JB (1981) Size of liver particles emptied from the human stomach. Gastroenterology 80:1489-1496

Mitchell C, Johnston R (2000) Pulsating suction during vacuum dewatering and its effect on the rate and extent of water removal. 54th Appita Annual Conference, Carlton, pp 443-447

Nagengast FM, Grubben M, Van Munster IP (1995) Role of bile acids in colorectal carcinogenesis. Eur J Cancer 31:1067-1070

Newitt PDM, Conway-Jones JM (1958) A contribution to the theory and practice of granulation. Chem Eng Res Design 36:422-442

Ottino JM (1989) The kinematics of mixing: Stretching, chaos, and transport. Cambridge University Press, Cambridge

Painter NS, Burkitt DP (1971) Diverticular disease of the colon: A deficiency disease of western civilization. Br Med J 2:450-454

Painter NS, Burkitt DP (1975) Diverticular disease of the colon, a 20th century problem. Clin Gastroenterol 4:3-21

Painter NS, Truelove SC (1964) The intraluminal pressure patterns in diverticulosis of the colon: Part i resting patterns of pressure. Gut 5:201-207

Painter NS, Truelove SC, Ardran GM, Tuckey M (1965) Segmentation and the localization of intraluminal pressures in the human colon, with special reference to the pathogenesis of colonic diverticula. Gastroenterology 49:169-177

Penry DL, Jumars PA (1986) Chemical reactor analysis and optimal digestion. Bioscience 36:310-315

Perez F, Accarino A, Azpiroz F, Quiroga S, Malagelada JR (2007) Gas distribution within the human gut: Effect of meals. Am J Gastroenterol 102:842-849

Poslinski AJ, Ryan ME, Gupta RK, Seshadri SG, Frechette FJ (1988) Rheological behavior of filled polymeric systems i. Yield stress and shear-thinning effects. J Rheol 32:703-735

Proano M, Camilleri M, Phillips SF, Brown ML, Thomforde GM (1990) Transit of solids through the human colon: Regional quantification in the unprepared bowel. Am J Physiol 258:G856-G862

Przybysz K, Szwarcsztajn E (1973) Effect of crill on pulp freeness. Przeglad Papier 29:105-108

Räisänen K (1996) High-vacuum dewatering on a paper machine wire section: A literature review. Paperi ja puu 78:113-120

Rauch R, Sangl R (2000) Latest findings on entrained air and dissolved gases in pulp suspensions. TAPPI 2000 Papermakers Conference. TAPPI Press, Atlanta, pp 159-175

Reppas C, Dressman JB (1992) Viscosity modulates blood glucose response to nutrient solutions in dogs. Diabetes Res Clin Pract 17:81-88

Roberts FG, Smith HA, Low AG, Ellis PR, Morris ER, Sambrook IE (1989) Influence of guar gum flour of different molecular weights on viscosity of jejunal digesta in the pig. Proc Nutr Soc 49:53A

Robertson J, Murison S, Chesson A (1992) Particle size distribution and solubility of dietary fibre in swede- (*brassica napus*) based and wheat-bran-based diets during gastrointestinal transit in the pig. J Sci Food Agric 58:197-205

Rogers SS, Van Der Walle C, Waigh TA (2008) Microrheology of bacterial biofilms in vitro: *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Langmuir 24:13549-13555

Ross-Murphy SB (1995) Structure–property relationships in food biopolymer gels and solutions. J Rheol 39:1451-1463

Rumpf H (1962) The strength of granules and agglomerates. In: Knepper WA (ed) Agglomeration. Interscience, New York, pp 379–418

Sakaguchi E (2003) Digestive strategies of small hindgut fermenters. Anim Sci J 74:327-337

Sampson WW, Kropholler HW (1995) Batch-drainage curves for pulp characterization. Part 1: Experimental. Tappi J 78:145-151

Schemann M, Ehrlein HJ (1986) Postprandial patterns of canine jejunal motility and transit of luminal content. Gastroenterology 90:991-1000

Schwartzberg HG (1997) Expression of fluid from biological solids. Sep Purif Rev 26:1-213

Sibley RM, Calow P (1986) Physiological ecology of animals: An evolutionary approach. Blackwells, Oxford

Siegle ML, Ehrlein HJ (1988) Digestive motor patterns and transit of luminal contents in canine ileum. Am J Physiol 254:G552-559

Singh BN, Kim KH (2000) Floating drug delivery systems: An approach to oral controlled drug delivery via gastric retention. J Controlled Release 63:235-259

Sperber I, Björnhag G, Ridderstråle Y (1983) Function of proximal colon in lemming and rat. Swed J Agr Res 13:243-256

Stanbury PF, Whitaker A, Hall SJ (1995) Principles of fermentation technology. Butterworth Heinemann, Oxford

Steadman R, Luner P (1985) The effect of wet fibre flexibility on sheet apparent density. In: Punton V (ed) Papermaking raw materials. MEP, London, pp 311-337

Steffe JF (1996) Rheological methods in food process engineering. Freeman Press, East Lancing

Stevens CE, Hume ID (1995) Comparative physiology of the vertebrate digestive system. Cambridge University Press, New York

Stickel JJ, Powell RL (2005) Fluid mechanics and rheology of dense suspensions. Ann Rev Fluid Mech 37:129-149

Stoodley P, Boyle JD, Lappin-Scott HM (2000) Influence of flow on the structure of bacterial biofilms. In: Bell CR, Brylinsky M, Johnson-Green PC (eds) Microbial Biosystems: New Frontiers : Proceedings of the 8th International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, pp 263-269

Stoodley P, Lewandowski Z, Boyle JD, Lappin-Scott HM (1999) Structural deformation of bacterial biofilms caused by short-term fluctuations in fluid shear: An in situ investigation of biofilm rheology. Biotechnol Bioeng 65:83-92

Suarez F, Furne J, Springfield J, Levitt M (1997) Insights into human colonic physiology obtained from the study of flatus composition. Am J Physiol 272:G1028-1033

Suarez FL, Levitt MD (2000) An understanding of excessive intestinal gas. Curr Gastroenterol Rep 2:413-419

Takahashi T, Goto M, Sakata T (2004) Viscoelastic properties of the small intestinal and caecal contents of the chicken. Br J Nutr 91:867-872

Takahashi T, Sakaguchi E (2006) Transport of bacteria across and along the large intestinal lumen of guinea pigs. J Comp Physiol B176:173-178

Takahashi T, Sakata T (2002) Large particles increase viscosity and yield stress of pig cecal contents without changing basic viscoelastic properties. J Nutr 132:1026-1030

Takahashi T, Sakata T (2004) Viscous properties of pig cecal contents and the contribution of solid particles to viscosity. Nutrition 20:377-382

Tatsumi D, Ishioka S, Matsumoto T (2002) Effect of fiber concentration and axial ratio on the rheological properties of cellulose fiber suspensions. J Soc Rheol Japan 30:27-32

Terrones G, Gauglitz PA (2003) Deformation of a spherical bubble in soft solid media under external pressure. Q J Mech Appl Math 56:513-525

Trotman IF, Misiewicz JJ (1988) Sigmoid motility in diverticular disease and the irritable bowel syndrome. Br Med J 29:218-222

Van Munster IP, Tangerman A, De Haan AFJ, Nagengast FM (1993) A new method for the determination of the cytotoxicity of bile acids and aqueous phase of stool: The effect of calcium. Eur J Clin Invest 23:773-773

Vincent JFV (1990) Fracture properties of plants. Adv Bot Res 17:235–287

Wakeman RJ, Tarleton ES (1999) Filtration: Equipment selection, modelling and process simulation. Elsevier, Oxford

Wildfong VJ, Genco JM, Shands JA, Bousfield DW (2000) Filtration mechanics of sheet forming. Part ii: Influence of fine material and compression. J Pulp & Paper Sci 26:280-283

Wood PJ, Braaten JT, Scott FW, Riedel KD, Wolynetz MS, Collins MW (1994) Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Br J Nutr 72:731-743

Ymele-Leki P, Ross JM (2007) Erosion from *Staphylococcus aureus* biofilms grown under physiologically relevant fluid shear forces yields bacterial cells with reduced avidity to collagen. Appl Environ Microbiol 73:1834-1841

York P, Row RC (1994) Monitoring granulation size enlargement process using mixer torque rheometry. First International Particle Technology Forum, Denver, pp 225-230

Zhou Z, Scales PJ, Boger DV (2001) Chemical and physical control of the rheology of concentrated metal oxide suspensions. Chem Eng Sci 56:2901-2920

Part II
The Effects of Contractile Activity of the Gut Wall and Its Structure on the Mixing and Absorption of Digesta

The second part of this book deals with the effects of contractile activity on the contents of the gut and on factors that limit transit of nutrients through the gut mucosa.

A body of work indicates that the characteristics and timing of contractile activity vary with the physical characteristics of the contents and with the digestive strategy of the segment of gut in which it occurs. Moreover, the pattern of the contractile activity in a particular segment may vary temporally so that different types of activity occur at particular times, notably in relation to the consumption of meals. Again contractile activity can occur locally or be distributed over a greater length of gut.

The remarkable variation in the morphology of the various compartments of the gut with species and with diet (Stevens and Hume 2004) is testament to the fact that the transit of digesta through particular segments of the gut varies with the chemical and physical properties of the diet and with the discontinuous nature of feeding. Hence contractile activity, mixing and residence time may all vary with diet and with the timing of feeding so as to maximise digestive efficiency and to avoid dangerous local anomalies of onflow. Contractile activity is known to vary locally, hence flow and mixing must also vary at a local level according to local hydrodynamic conditions within the lumen. However, contractile activity, flow and mixing may also vary at a segmental level (Gregersen 2003) being synchronised by ascending and descending elements of the enteric nervous system according to information received from proximal and distal sites, and from the central nervous system (Bornstein et al. 2002). Hence these regulatory elements may modulate contractile dynamics within an entire segment of gut by influencing the frequency, distance and direction of propagation of local contractile events via the migrating motor complex cycle or MMC (see below), by controlling local or overall tone to modulate capacity and by directly regulating flow through functionally discrete constricting regions at the commencement or termination of the segment, i.e. sphincters.

A body of work is accumulating showing that the gut wall is adapted not only to maximise the absorption of nutrients but also to present a barrier to the transit of potentially pathogenic bacteria (Farhadi et al. 2003; Lamont 1992; Newberry and Lorenz 2005; Yuan and Walker 2004) potentially antigenic material (Vogelsang et al. 2000) and enable cross-talk with potentially beneficial microorganisms to modulate immune response (Kelly et al. 2003; Kelly et al. 2005). The structural and physiological specialisations associated with such functions are likely to complicate the optimisation of mass transfer. Hence it is necessary to critically review what is known regarding the absorptive capability of the various components that comprise or are associated with the gut mucosa rather than assuming a general principal of optimisation of mass transfer.

In this section we shall first describe the general characteristics of the contractile activities that can occur in the wall of the gut, provide an overview of their possible effects on lumen contents and a description of the manner in which they are generated. The latter will pay particular attention to local regulation in view of the need to prevent blockage and maintain onflow in the face of local inhomogeneity of the contents. In a subsequent chapter we will review the manner in which these activities vary in various segments of the gut and effect flow between segments. In a further chapter we will describe the manner in which contractile activities operate within particular segments of the gut and govern local flow within the segment. In the final chapter we will review what is known regarding the influence of the various layers associated with the mucosa on mass transfer of nutrients.

References

Bornstein JC, Furness JB, Kunze WAA, Bertrand PP (2002) Enteric Reflexes that Influence Motility. In: Brookes SJH, Costa M (eds) Innervation of the Gastrointestinal Tract. Taylor & Francis, London

Farhadi A, Banan A, Fields J, Keshavarzian A (2003) Intestinal barrier: an interface between health and disease. J Gastroenterol Hepatol 18:479-497

Gregersen H (2003) Biomechanics of the gastrointestinal tract: new perspectives in motility research and diagnostics. Springer Verlag, New York

Kelly D, Campbell J, King T, Grant G, Jansson E, Coutts A, Pettersson S, Conway S (2003) Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR- and RelA. Nature immunology 5:104-112

Kelly D, Conway S, Aminov R (2005) Commensal gut bacteria: mechanisms of immune modulation. Trends in immunology 26:326-333

Lamont J (1992) Mucus: the front line of intestinal mucosal defense. Ann N Y Acad Sci 664:190-201

Newberry R, Lorenz R (2005) Organizing a mucosal defense. Immunol Rev 206:6-21

Stevens C, Hume I (2004) Comparative physiology of the vertebrate digestive system. Cambridge Univ Pr

Vogelsang H, Schwarzenhofer M, Oberhuber G (2000) Changes in gastrointestinal permeability in celiac disease. Dig Dis 16:333-336

Yuan Q, Walker W (2004) Innate immunity of the gut: mucosal defense in health and disease. J Pediatr Gastroenterol Nutr 38:463

Chapter 7
Contractile Activity and Control of the Physical Process of Digestion Within a Gut Segment

Contents

7.1 General Aspects of Contractile Activity in the Wall of the Gastrointestinal Tract

The smooth muscle in the wall of the gut is of ‘unitary type’, which contracts spontaneously in the absence of neural and endocrine influence, and can contract in response to stretch. The neuronal elements, which associate with smooth muscle cells and modulate their activity, are not linked directly to smooth muscle cells via motor end plates Thus nerve–muscle distances are large and the transmitters released by such neuronal elements must diffuse over large distances in order to influence large numbers of smooth muscle cells (Wood 2006). However, two morphological

R. G. Lentle, P. W. M. Janssen, *The Physical Processes of Digestion*,
DOI 10.1007/978-1-4419-9449-3_7,

features serve to secure smooth integration of neuronal and local regulation of contractile activity. Neuronal end structures are in close association with interstitial cells of Cajal (ICCs), which in turn are linked with smooth muscle cells via gap junctions. This structure allows the effect of pacemaker potentials generated by ICCs to be integrated with neuronal inputs and relayed to smooth muscle cells. Individual smooth muscle cells are also linked with each other by similar gap junctions enabling locally generated action potentials to propagate between them. This unique structure allows for smooth integration of contractile activity in the longitudinal and circular elements of the gut wall according to local, regional and central requirements.

Contractile activity in the wall of the intestine may be episodic, i.e. phasic in nature, where the intensity of the contractile event rises to a peak and subsequently subsides. Alternately, the activity may be maintained over a longer period of time, i.e. tonic. Such tonic contraction may continue while other contractile elements undergo phasic contractile activity. The maintenance of a degree of tonic activity in the circular muscle of the wall of a tubular segment of gut would cause the volume of the lumen to be reduced in proportion to the degree of muscle shortening and may reduce the capacity of the segment, always provided that there was little resistance to compression by the contents of the lumen, and that the constriction did not lead to compensatory lengthening of the segment. Similarly, widespread tonic contraction of longitudinal muscle in the wall of a tubular segment of gut could lead to its shortening and a compensatory increase in the diameter of the lumen, where there was resistance to the outflow of contained digesta. Phasic contraction of circular muscles in a localised section of the segment would lead to displacement of the contents to the adjacent section, provided it was sufficiently elastic to receive the displaced material. If it acted in a section adjacent to one in which there was local phasic circular muscle contraction, phasic contraction of longitudinal muscle would cause local expansion and accommodation of contents from adjacent regions of the lumen, always provided the contents were sufficiently fluid and the walls of the section were sufficiently compliant. Given that in some situations the section of phasic contraction may move either proximally or distally along the tubular segment to cause digesta to be displaced to an adjacent segment of gut, it can be seen that the organisation of intestinal musculature allows displacement of the contents of a segment by either phasic or tonic contractions. Further, both mechanisms may lead to mixing, either locally, or as displaced material moves between successive segments.

This is not to say that phasic and tonic contractions operate independent of each other. The level of tonic contraction in the wall of a segment may influence the loading of tension receptors and hence the ease with which a phasic contraction is triggered (Spencer et al. 2001). Again the hydrodynamic effects of a combined tonic and phasic contraction will depend to some extent on the nature on the morphology of the segment. Hence in segments with a sacculate form, such as the stomach (see Chap. 9), a general increase in tone may bring about a closer approach of opposing walls during phasic contraction but is less likely to bring about full occlusion of the lumen than in a tubiform segment. Thus a phasic superimposed on a tonic contraction is more likely to induce mixing in a sacculate segment and more likely to induce propulsion in a tubiform segment.

The operation of sphincters, which restrict the transfer of displaced material between adjacent segments, may influence not only the displacement of material but also the extent to which the operation of radial and longitudinal muscles can be accompanied by shortening, given that the contained material is liquid and therefore incompressible. Hence the operation of sphincters may influence mixing and backflow, particularly in portions of a segment that lie in close proximity to them and are full of digesta. Consequently, even though we consider aspects of local and segmental control of flow and mixing separately in this section of the book, it is important to understand that their final effects are interrelated, a distally moving constricting phasic contraction may lead either to onflow or to mixing according to the state of an adjacent sphincter and the state of the succeeding segment.

7.1.1 The Triggering and Maintenance of Phasic Contractile Activity

There are a number of hypotheses regarding the means by which phasic contractile activity is triggered and maintained, notably regarding the role of the luminal contents and their physical properties. One view holds that peristalsis results from the action of ascending excitatory and descending inhibitory reflexes, which may be initiated and maintained by the presence of gut contents and modulated according to their physical and chemical properties. Hence we may expect to see ongoing local summation of a number of stimuli, perhaps at the junctions of neuronal territories, being progressively triggered as the bolus traverses the length of the small intestine. Conversely, if peristalsis comprises a stereotyped, self-perpetuating, neuronal reflex, which continues independently of gut contents after its initiation, its passage will on occasion become uncoordinated with that of the initiating bolus, e.g. overtaking it, a phenomenon that has been observed by a number of authors (Bornstein et al. 2002; Spencer et al. 1999). The latter 'all or none' (Kosterlitz and Lees 1964) hypothesis is also favoured by work showing that circular muscle contraction evoked by circumferential stretch continues along an opened segment of small intestine, i.e. traverses a section of gut where it cannot continue to be propagated by distension from fluid flow and pressure (Spencer et al. 1999). However, other work suggests that localised stimuli lead to graded response in myenteric neurones and smooth muscle (Brookes et al. 1999; Spencer et al. 1999), that muscle tone and contraction are important not only for initiation but also for maintenance of peristalsis (Spencer et al. 2001), and that the activity of sensory neurones is dependent upon the contraction and tone of smooth muscle (Kunze et al. 1999). Hence while the propagation of peristalsis continues across an opened segment of small intestine and the velocity of propagation is not appreciably modified by fluid distension, its amplitude tends to decline in the absence of ongoing distension (Spencer et al. 2001).

It is conceivable that the propagation of peristalsis may be driven by a 'single' set of sensors, for example, the unloading of a stretch-induced inhibitory sensory input into an interneuron by the initiation of contraction allows inhibition to be turned off and contraction of the next 'ring' of circular muscle to be activated (Waterman et al. 1994). However, in such a system, the speed at which the contents are displaced into the succeeding segments will govern the speed at which inhibition is removed, and hence the speed of sequential activation of each ring of muscle. In this case, the viscosity of the chyme will influence the velocity of peristalsis. There is limited evidence for this. While significant differences are reported between the speeds of peristalsis of boluses of air from those of particulate suspensions, no significant differences are reported between the speeds of peristalsis of boluses of oil and those of water (Larson and Schulze 2002), or between boluses of guar gum solution and those of watery solution (Lentle et al. 2007).

Information regarding the physical nature of the contents of a segment of intestine may be obtained via a number of modalities. The pattern of distension, i.e. the activation state of a series of tension or length receptors arrayed along the longitudinal axis of a length of intestine, could serve to distinguish the more localised distension of a solid bolus from the more widespread distension when the contents are watery. Also the speed at which tension receptors are unloaded in adjacent segments may allow the flow properties of the contents to be distinguished. The operation of such processes requires the integration of sensory information from adjacent sensors. While there is no direct evidence of such processes influencing neuronal activation within the enteric nervous system (ENS), work using fluids of different rheological properties suggests it may occur. For instance, the direction of gastric peristalsis is reversed when the ex vivo rat stomach is perfused with material with an apparent viscosity that lies within the range of that of normal gastric chyme but which exhibits extensional flow and shear thickening properties such that the patterns of tension in the constricting and receiving sections are likely to differ from those encountered with normal chyme (Lentle et al. 2010).

7.1.2 Tonic Contractile Activity

At most times, intestinal contractile activity comprises a combination of phasic and tonic activity (Gregersen 2003). Hence the form, amplitude, speed of progression, and frequency of phasic intestinal contractions, as well as the capacity and distensibility (Gregersen 2003) of the receiving or delivering segment (i.e. tone) of the intestine must all be appropriately modulated to ensure optimum flow, mixing and digestive efficiency.

The interplay between phasic and tonic activity is of critical importance in the regulation of digestive efficiency. A number of vertebrate species, such as macropods, are able to adjust their intake to compensate for a reduction in nutritional density but still maintain the same transit time (Dellow 1982). Such a strategy may be achieved either by reducing tone so as to increase the cross-sectional area of some or all of the gut, or in situations where the lumen of the gut is not completely filled with digesta, by phasic activity, i.e. by increasing the frequency of antiperistaltic phasic contractions (Stevens and Hume 1995). The extent to which these two mechanisms contribute to compensation for variation in the nutritional density of food intake in humans is not known.

The modulation of tonic and phasic contractile activity may result either from the inbuilt characteristics of the contractile machinery or from feedback from local mechanoreceptors. Recent findings showing that the degree of tonic contraction and the frequency and amplitude of phasic contractile activity in the gastric antrum are curvilinearly related to distension volume have prompted the hypothesis that local change in the length–tension relationship in smooth muscle may fine tune contractile response (Gregersen et al. 2005). Hence, within certain limits, greater distension would increase the degree of overlap of the contractile elements of smooth muscle in the wall of the gastric antrum causing the contractile activity to be augmented in a manner that is broadly analogous to that in cardiac muscle, i.e. the operation of Starling's law.

7.1.3 Mechanoreceptors

Less localised modulation of contractile activity than that resulting from the direct effects of stretch on contractile apparatus may be initiated by stretch receptors associated with the neuronal (Gregersen and Kassab 1996) or cellular elements within the gut wall. A number of reports indicate that some types of intrinsic primary afferent neurones (IPANS) may be sensitive to distension (Johnson et al. 1998; Johnson et al. 1996), and more recent work indicates that ICCs are also mechanosensitive (Strege et al. 2003) and respond directly to distension by increasing the amplitude but not the frequency of their slow-wave discharge (Wang et al. 2010).

The initiation and modulation of contractile activity may be mediated by a number of types of mechanoreceptors, the relative roles and sensitivities of which are currently unknown. Thus mechanoreceptors and associated elements of the vagus nerve have been identified in the myenteric plexus of the oesophageal wall (Zagorodnyuk et al. 2001) and small intestine (Song et al. 2009), but the physical stimulus to which they respond is subject to debate (Gregersen et al. 2005). Analysis of electrophysiological outputs from vagal and other afferent neurones (Andrews et al. 1980; Davison and Clarke 1988; Ozaki et al. 1999; Takeshima 1971) suggests there are two populations of mechanoreceptors (Fig. 7.1), those that respond to tension and act ‘in series’ with contractile elements in a similar manner to the Golgi mechanoreceptors found in skeletal muscle tendons, and those that respond to stretch, acting ‘in parallel’ with contractile elements so that they respond to changes in volume, i.e. distension (Tack et al. 2004).

The correlation of circumferential tension and stretch with transmural pressure has been explored by distending living sections of tubular gut, such as the human oesophagus (Barlow et al. 2002) and ileum (Gao et al. 2003), with balloons and

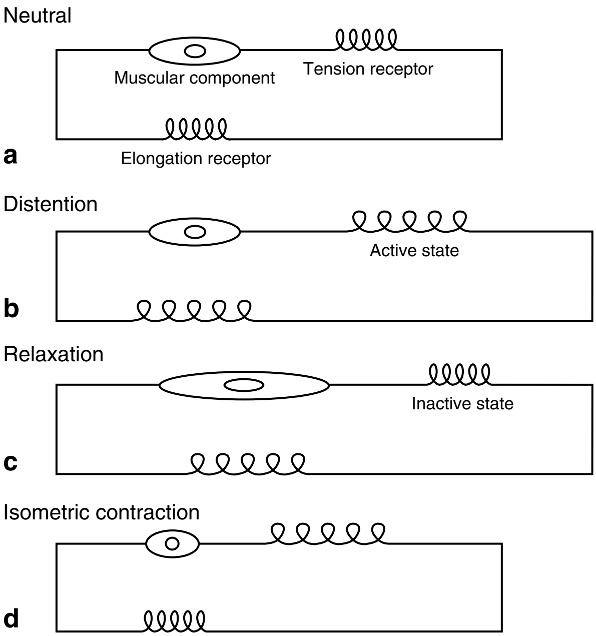


Fig. 7.1 Schematic illustration of the responses of tension mechanoreceptors (in series) and elongation mechanoreceptors (in parallel) to different stimuli (distension, relaxation, and isometric contraction). **a** Represents neutral condition. **b** Distension is accompanied by activation of both tension and elongation mechanoreceptors when muscular contraction status is unchanged. **c** Muscular relaxation is accompanied by inactivation of tension but not elongation receptors, and muscular component is lengthened. **d** Isometric contraction accompanied by activation of tension but not elongation receptors and the muscular component is shortened

using the law of Laplace to derive circumferentially vectored tension. The results of this work suggest that the principal arbiters of tone are mechanoreceptors that respond to radial stretch. However, the use of balloon distension may not be appropriate in structures such as the stomach (Gregersen and Kassab 1996; Zhao et al. 2005) whose walls exhibit anisotropy, i.e. their biomechanical properties vary with the direction in which force is applied.

The ease with which a section of the gut is distended, i.e. its inherent elasticity and superadded muscular tone, may influence flow and mixing within (Gregersen and Kassab 1996; Schulze-Delrieu 1991). When a section of gut is receiving digesta from an adjacent contracting section, the ease with which its walls distend will influence the rate at which pressure rises, and the rate of onflow (Kwiatek et al. 2009). For example, circumferential muscular tone is locally increased during the passage of phasic contractions through the gastric antrum (Gregersen et al. 2006) so that displaced digesta is less readily accommodated in the receiving segment and its axial displacement is continued (Fig. 7.2).

Measures of distensibility are to be preferred when examining the behaviour of the walls of the gut, because they exhibit a curvilinear relationship between tension

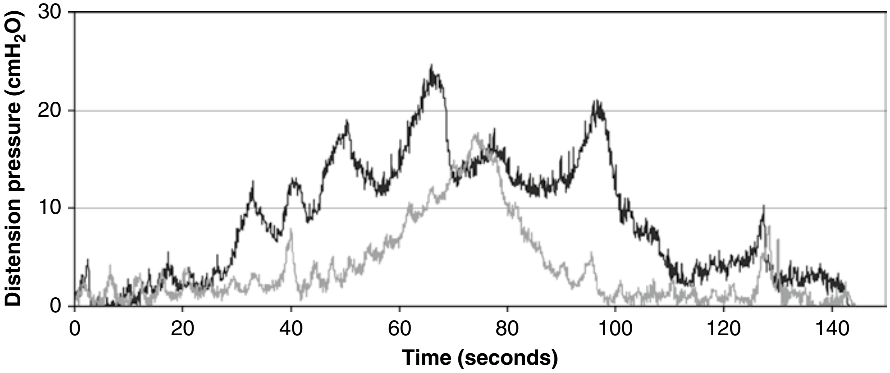


Fig. 7.2 Pressure within the human gastric antrum during a symmetrical ramped increase and decrease in volume. The *black* and *grey* tracings are from distension before and after the administration of hyoscine which inhibits the tonic contractile response. It is apparent that the gastric antrum exhibits both phasic and tonic contractions during distension, and that hyoscine decreases contractile activity. (Gregersen et al. 2006)

and strain (Gregersen and Kassab 1996) that is not completely described by the ratio of the increment in volume to that in pressure, i.e. compliance. The curvilinear form of the strain tension curve, with increasing resistance to strain at high wall tensions, is thought to result from the presence of inelastic collagen fibres, which uncrimp from their folded resting state under tension and become taught (Fackler et al. 1981; Orberg et al. 1983). The behaviour of these inelastic fibres, along with that of smooth muscle, renders the response of the gut wall to distension time dependent, i.e. viscoelastic. This time-dependant behaviour can be conceived mechanically as the outcome of an elastic element (a spring) coupled to a viscous element (a dashpot). Determination of distensibility prior to and following the addition of agents that specifically inhibit smooth muscle contraction in living tissue enables the contribution of muscular tone to be assessed and indicates that it has a significant influence on overall distensibility (Gregersen et al. 2006). Hence an appropriate alteration in smooth muscle tone may influence the speed of onflow in the antrum and the extent of mixing (Gregersen and Kassab 1996).

It is noteworthy that the collagen fibres and smooth muscle cells vary in their orientation in the wall of the gut so that phasic or tonic contraction will alter both radial and longitudinal dimensions. Hence the use of changes in radial diameter and wall thickness, as determined by ultrasonographic and other techniques (Mittal et al. 2005), as a basis for determining wall tension may be to some extent flawed, because they do not allow for simultaneous changes in longitudinal dimensions (Gregersen 2003).

Given that digesta generally behaves as a pseudoplastic fluid (Lentle and Janssen 2008), and that its apparent viscosity and pseudoplasticity varies with the diet (Dikeman and Fahey 2006), it is likely that information is required from arrays of both stretch and tension receptors to enable the forces generated in the contracting and receiving segments during peristalsis to be appropriately assessed. Hence, as the apparent viscosity (and hence ease of flow) of digesta varies with the rate at which shear is generated, and the relationship between shear rate and apparent viscosity varies with the diet, there is no 'standard curve' that can predict flow from a single assessment, either of circular tension or of stretch at a given point along the gut. However, if the rates of change of tension in the contracting segment and stretch in the receiving segments are compared, this information can be used to derive the rate at which shear force is applied so as to obtain the appropriate flow. Similarly, the simultaneous use of both tension and stretch receptors in either the constricting or the receiving segment to compare rates of change of stretch and tension would be equally effective and is analogous to rheometric evaluation. We can find no quantitative evidence that the rate, form or frequency of peristalsis changes with the physical properties of normal (pseudoplastic) digesta, hence adjustment of tonic contraction may be more important than that of phasic contraction in local control of flow. However, as noted earlier, the orderly progression of antral contractions is disrupted when the stomach is perfused with materials that have atypical rheometric properties, i.e. exhibit shear thickening and extensional flow (Lentle et al. 2010).

7.1.4 Control of the Physical Processes of Digestion at a Segmental Level

In this section, we shall consider mechanisms that control the physical process of digestion at a segmental level. These include the control of phasic and tonic contractile activity at a segmental level, notably in the postprandial and intermeal intervals (migrating motor complex (MMC) cycle), and the effects of morphology at junctions between succeeding segments and at junctions with secretory ducts. These effects may be modified according to local functional requirements and conditions. Following their broad description, we will consider the principal sphincteric and junctional regions separately. While these systems and sites of segmental control are considered separately, it is important to realise that they act in a coordinated manner in vivo. Thus the opening of a sphincter may be accompanied by modulation in the capacity of adjacent segments and may be promoted by neuronal events that modulate phasic and tonal contractile activity. For example, neuronal activity associated with phase III of the MMC cycle simultaneously influences contractile activity in the biliary system, the sphincter of Oddi, and the duodenum.

While it is likely that tonic, phasic and sphincteric contractile activity all influence flow and residence time within particular segments of gut, relatively little quantitative data regarding their relative contribution are available. After over a century of study, much is known of the electro- and pharmaco-physiology of the enteric and autonomic nervous system, and of the factors inducing feedforward and feedback regulation (Brookes and Costa 2002; Johnson 2006). Conversely, little is known of the quantitative aspects of such regulation and, in particular, regarding the role of tone in regulating pressure, volume, mixing and onflow in the various segments of the gut (Gregersen 2003).

7.1.4.1 The MMC Cycle: Fasting and Postprandial Periods of Motility

There is extensive evidence that the contractile activity of the gut undergoes temporal modulation that results in the establishment of distinctive patterns of motility during the fasting (interdigestive) and postprandial periods (Husebye 1999).

The fasting period is characterised by repeated cycles of activity, which each consist of three or four successive 'phases' with an overall cycle duration of about 85–110 min in humans, although the actual duration is highly variable both within and between subjects (Husebye 1999). The cycle of activity during the interdigestive period is termed the MMC cycle after the MMCs that occur as a regular sequence during phase III of the cycle. An MMC comprises a burst of oscillating electrophysiological activity and accompanying peristaltic contractions that slowly traverses a length of intestine. The slowly propagating MMC contractions occur at the frequency of local slow waves, each contraction starting slightly distal to the previous one as the complex migrates distad. It is noteworthy that slow waves (cyclical oscillations in the membrane resting potential generated in ICC) (Sanders et al. 2006) may be modulated according to local mechanosensory input (Thuneberg 1999; Timmermans 2001). Hence the frequency of MMC-induced contractions may be modulated by lumen conditions that bring about mechanosensory stimulation.

In humans, MMCs are thought to originate principally in sites from the stomach to the proximal ileum (Kellow et al. 1986), indeed, when the small intestine is subdivided into successive segments, each segment develops an inherent MMC (Sarna et al. 1983). The oscillation frequency of these spontaneously generated MMCs tends to be higher in more proximal sites, and hence MMCs migrating from proximal sites entrain more distal sites. The extent of distal propagation of many MMCs is limited, and while many propagate as far as the proximal ileum, few reach the distal ileum (Kellow et al. 1986).

Phase I of the MMC cycle is characterised by electrophysiological and contractile quiescence, while phase II is characterised by irregular bursts of electrophysiological and contractile activity. Regular MMC electrophysiological and contractile activity only appears during phase III. The majority of phase III MMCs originate in the stomach (Dooley et al. 1992) with greater numbers arising at this site after longer periods of fasting (Wilmer et al. 1993). In some species, a further short period of more intermittent contractile activity occurs, which is termed phase IV (Romański 2009). The proportions of the MMC cycle that are occupied by the different phases vary greatly between subjects and species (Romański 2009). Phases I and II occupy the majority of the MMC cycle, roughly in equal proportions, while phase III occupies 5–10% of the cycle.

In the small intestine, MMCs mainly propagate very slowly distad with estimated velocities of 5–10 cm/min in the proximal jejunum decreasing to 0.5–1.0 cm/min in the distal ileum (Kellow et al. 1986). Consequently, the three phases of the cycle may occur at the same time, each at a different point along the length of the gut, e.g. a phase III episode may commence in the duodenum while the distal ileum is still undergoing phase II activity (Costa et al. 2005). It is also noteworthy that the direction of propagation of MMCs may vary in some segments, notably the colon (Sarna

1985). In this case, the site of the highest frequency of slow-wave oscillation, i.e. the dominant pacemaker, differs correspondingly (Pluja et al. 2001), being situated not at the orad border of the colon (Elliott and Barclay-Smith 1904; Hukuhara and Neya 1968) but in the mid-colon (Elliott and Barclay-Smith 1904). More recent work suggests that the location of the colonic pacemaker may vary according to a combination of myogenic and neurogenic factors (Hennig et al. 2010; Powell and Bywater 2001).

The temporal pattern of change in the phase of the MMC cycle may vary between gut segments, possibly in relation to their mode of operation. Hence, in compartments such as the stomach that act broadly as batch reactors (Penry and Jumars 1987), the various phases of the MMC cycle commence synchronously in the various segments, i.e. the fundus, corpus and antrum. In others, such as the small intestine that act broadly in the manner of continuous plug flow reactors (Penry and Jumars 1987), the site of change in MMC pattern migrates caudally (Sarna et al. 1985). The timing of the switch in MMC phase may vary between adjacent compartments according to local functional activity, e.g. the gastric phase III activity invariably starts before that in the duodenum but ends synchronously in these two segments (Sarna 1985). The concurrence of MMCs in some functionally related components of the digestive tract allows their action to be coordinated, e.g. periods of phasic and tonic activity in the lower oesophageal sphincter are correlated with the phases of MMC activity in the stomach (Holloway et al. 1987; Itoh et al. 1978). Similarly, the pattern of contractile activity in the sphincter of Oddi is synchronised with the phases of MMC activity in the duodenum (Yokohata and Tanaka 2000).

In the small intestine, the irregular contractile events that occur during phase II of the MMC cycle are thought to facilitate mixing and absorption. Thus the duodenal absorptive flux is twice as high as the secretory flux during periods of lower motor activity (phases I–II) but is four times as high during periods of higher activity phases II–III (Mellander et al. 2001). The relatively short period of high-intensity contractions, which comprises phase III, has been termed the housekeeper phase and is hypothesised to clear indigestible residues from the various segments of gut (Code and Schlegel 1973).

The lack of correlation between the timing of MMCs in the colon with those in the proximal or distal end of the small intestine suggests that MMCs arise within the colon rather than propagate into it from the small intestine (Sarna et al. 1984). This is consistent with work showing that the sites at which the spontaneously regenerating slow waves, which synchronise smooth muscle contraction, are generated differs in the colon from that in the small intestine. In particular, slow waves originate in the submucosa of the colon (Sanders et al. 1990) rather than in the area between the circular and longitudinal muscle layer, i.e. originate in the ICC-SM rather than the ICC-MY (Pluja et al. 2001). However, some authorities suggest that MMC-like activity migrates distally through both plexi (Sarna 1985).

In carnivores and omnivores, the ingestion of food causes the MMC cycle to be interrupted and induces a postprandial pattern of activity characterised by irregular electrophysiological activity, and the onset of segmentative contractions and short peristaltic waves. The postprandial period continues for 3–10 h, depending on caloric intake, and is said to terminate when the phase III of a new MMC cycle appears. This definition arises from difficulties in distinguishing postprandial electrophysiological activity from that which occurs during phase II of the MMC cycle.

The institution and maintenance of the postprandial period are thought to result principally from overriding vagal activity. Cooling of the vagus nerve can prevent the initiation of the postprandial period after feeding and can terminate an existing postprandial period (Hall et al. 1986). However, while the MMC cycle is also interrupted immediately following division of the vagus nerve (vagotomy), it is subsequently reinstated although with some departure from the typical pattern (Hashmonai et al. 1987a; Marik and Code 1975).

The incidence of MMCs is also influenced by a number of humoral factors, in particular, they are induced by the secretion of motilin from the duodenal mucosa (Hashmonai et al. 1987b; Heitz et al. 1978). In essence, the MMC cycle is thought to originate in the intrinsic nervous system but to be modulated by the extrinsic nervous system, hence the postprandial period results from the suppression of the MMC cycle by the extrinsic nervous system (Husebye 1999).

MMC cycles have not been convincingly recorded from ex vivo tissue preparations (Bornstein et al. 2002). However, the pattern of activity seen in some ex vivo preparations has been said to have some of the characteristic properties of MMC phase III activity.

7.1.4.2 Other Segmental Types of Regulatory Rhythms

Other types of episodic rhythms that govern the activity of lengths of intestine have been reported. Two types of contractile activity that are distinguishable from MMCs, termed discrete clustered contractions (DCCs) and prolonged propagated contractions (PPCs), appear episodically in the terminal ileum (Quigley et al. 1984). PPCs are rapidly propagating, isolated, high-amplitude waves, which are of longer duration than the regional slow waves. DCCs are series of short bursts of rhythmic phasic contractions, which also propagate rapidly through the terminal ileum. Both types of contraction have propulsive capability and are thought to contribute to emptying of the terminal ileum (Kruis et al. 1985). These activities coincide with periods of reduction in the tone of the ileocolic sphincter, indicating that there may be functional coordination between the two (Dinning et al. 1999). Hence, it may be inappropriate to consider such regional activity as a separate means of temporal regulation of gut activity and better to consider it as an aspect of sphincteric control.

7.2 General Effects of Junctional Morphology

When considering the action of sphincters, it is important to note that they are frequently situated at junctions between segments of the gut that differ in their morphology. Hence, apart from the effects of any localised phasic or tonic contractile activity, the transitional morphology between the adjoining segments can influence the characteristics of flow between them.

A further consideration arises from the fact that digesta generally comprises a suspension of particulate matter. In some situations the particulate and liquid components may behave differently when digesta is flowing though the morphological constraints of a junction.

7.2.1 Junctional Morphology and the Flow of Digesta

Bernouelli's theorem indicates that the velocity of watery digesta will increase as it flows through narrowing structures such as the pyloric canal, and the fluid pressure within it will fall relative to that in the wider proximal portion. Hence the pressure will be correspondingly lower in the jet that emerges from such a narrowed structure and enters a wider lumen, such as that of the duodenum. As the rate of flow will be lower in the wider bore of the duodenum, and fluid pressure correspondingly higher, the duodenal fluid surrounding the emerging jet will tend to flow towards it, becoming entrained and admixed with it, in a manner analogous with that occurring in a water-jet eductor.

Similarly at a T-junction, there will be more efficient admixture of the contents of a wider tube with contents emerging from the abutting narrower tube when the latter are flowing at a higher velocity. Such admixture may be aided by adaptations that serve to augment the velocity of inflow from the narrower tube. For instance, the formation of a reservoir at the distal end of the narrower tube that intermittently and forcibly ejects its contents into the larger tube will facilitate mixing. Such an adaptation is seen at the ileocaecal junction of the rabbit where a muscular-walled sacculate reservoir, the sacculus rotundus (Snipes 1979), contracts at intervals to force its contents into the caecum (Ehrlein and Ruoff 1982). The sacculus contracts with greater amplitude when caecal 'antiperistaltic waves', contractile waves that have travelled from the proximal end of the caecum, increase caecal pressure in the region of the ileocecal junction (Ehrlein and Ruoff 1982). The increase in amplitude presumably maintains forcible inflow of the jet into the caecum in the face of the higher pressure surrounding it.

Similar effects in increasing velocity of inflow of digesta from a narrower to a larger lumen may be obtained by augmenting the motility of the narrower tube proximal to the point of its entry into the larger structure. Thus in the terminal ileum of the pig, giant migrating contractions occur more frequently than in man (Cuche and Malbert 1998). These contractions have a high velocity and hence are able to propel fluid ileal digesta more forcibly through the ileocaecal junction into the caecum (Hipper and Ehrlein 2001). In humans, digesta accumulates in the distal ileum and its entry into the caecum is likely to be augmented by the forceful ileal PPC and DCC contractions described earlier (Dinning et al. 2008).

7.2.2 *Differential Flow of Particulate Suspensions*

The movement of particulate suspensions through a junction between two gut segments or through a narrowed sphincter is influenced by the change in wall contour across the structure (Lentle et al. 2009). Where digesta is flowing from a larger to a smaller diameter compartment, the speed of flow will increase and contained particles will tend to approach each other as flow lines converge, thus increasing the likelihood that they will collide and entangle to form a mat. Subsequent flow of liquid through the mat will entrap more suspended particles causing them to accumulate and bring about ongoing local separation of the liquid from the solid phase. Conversely, where digesta flows from a smaller to a larger diameter compartment, velocity will decrease and the contained particles will move apart as flow lines diverge. Therefore, we may expect to see morphological adaptation to such contours at sites where mat formation or mat dispersal are required. For example, during colonic contraction, the narrowing of the necks of avian caeca at their point of union with the larger diameter colon will promote the matting of larger particles of colonic digesta so that only the fluid phase of digesta and contained fine particulates flow on into the caeca (Lentle et al. 2009). Conversely, the divergent flow lines of chyme that has been retropulsed from the narrowed distal antrum into the wider proximal antrum and body of the stomach will cause the particulate matrix of any contained fragments of food boluses to be dispersed.

7.3 Sphincteric Control

Sphincters provide a means of controlling the transit of digesta through successive segments of the digestive tract, and hence may act to retain any digesta that is in need of further processing in a more proximal segment. Early work indicated that sphincters modulated digesta transit by tonic contraction, the extent of contraction of circular muscle and consequent radial constriction serving to limit flow (Anuras et al. 1974; Conklin and Christensen 1975). The contractile activity of sphincteric regions resembles that of the gut wall, varying both in tonic and in phasic activity (Woods et al. 2005). Indeed, it has been suggested that the principal distinction between sphincters and other sites in the gut wall lies in their sensitivity to hormonal and other regulators rather than their mechanical activity (Gregersen 2003). However, it is important to note that while local changes in the tone and phasic activity of the sphincter in response to such regulation may contribute to the regulation of digesta transit, the latter may also be regulated by modulation of tonic and phasic activity in the segments adjacent to the sphincter.

The contribution of phasic contractions to sphincteric control has not been fully elucidated. While the effects of phasic contractions on flow dynamic in the pylorus are beginning to be understood (see below), we can only hypothesise as to their effects in the lower oesophageal sphincter (Dent et al. 1983) and the sphincter of Oddi (Yokohata and Tanaka 2000), which both exhibit rhythmic contractions, some of which are coordinated with phase III MMCs. It is possible that these phasic contractions serve a 'housekeeping' function, i.e. aid in the expulsion and onflow of any impacted or otherwise accumulated solid matter (Code and Schlegel 1973; Yokohata and Tanaka 2000). In investigating such function, it is important to note that particular aspects of sphincteric function may vary between species, and hence animal models may not always be useful in predicting flow dynamics at the same site in humans. For example, cyclic contractile activity in the sphincter of Oddi in humans is proposed to restrict the onflow of bile contained in and pressurised by the elastic walls of the biliary tubular system (Woods et al. 2005), while in the dog and a number of other species, the same sphincter actively pumps bile from the biliary system to the duodenum (Sand et al. 1997).

Recent evidence suggests that there are considerable differences in the morphology and physiology of the various sphincters. Differences in flow requirements and in the physical characteristics of the contained material may dictate the physical means by which the sphincteric lumen is occluded. In sphincters at sites where flow must be strictly unidirectional, e.g. the sphincter of Oddi (Bosch and Pena 2007), and where the contained fluid is Newtonian in nature (Ooi et al. 2004) and free from any particulate material, then simple radial occlusion such as occurs in the occlusive part of this sphincter (Barraya et al. 1971) may suffice. At other sites where the transiting material contains significant amounts of particulate matter, e.g. the pylorus, occlusion by prolapsing folds of mucosa (Ramkumar and Schulze 2005) may be better able to withstand defeat by impacted or matted particulate material. Where it is important to restrict the aggregation of particles or backflow of any expressed liquid phase, e.g. the upper (Singh and Hamdy 2005) and lower oesophageal sphincters, there are higher levels of morphological and physiological complexity with integration of the action of extrinsic pharyngeal (Singh and Hamdy 2005) and diaphragmatic musculature (Mittal and Balaban 1997) respectively, in order to modify the contour of the lumen wall within the sphincter and the pressure gradient across it.

It is noteworthy that sphincteric action may take place in the absence of any morphological adaptation. Either the tone or the frequency of phasic events in a localised segment of gut that is immediately adjacent to a segmental junction may be locally modified to act as a sphincter, as, for example, in the human ileocolic sphincter (Malbert 2005).

Given the range of local functional adaptations according to flow requirements and the rheological properties of digesta and between species, it is difficult to formulate any general rules and more useful to separately examine the mechanics of each sphincter. In the following section, we concentrate on the mechanical aspects of the various sphincters in the human gut and refrain from describing in detail the numerous neuronal, hormonal and other physiological stimuli that act at the different sites.

7.3.1 The Upper Oesophageal Sphincter (UOS): Safe Bolus Transit with Reduced Compression

Closure of the UOS establishes a zone of high intraluminal pressure, which isolates the lumen of the pharynx from that of the oesophagus preventing the entry of air into the oesophagus, and the reflux of digesta from the stomach and oesophagus into the laryngopharynx. The elevated pressure results from the high resting tone in the inferior pharyngeal constrictor, the cricopharyngeus and the cranial-most section of the circular muscle of the oesophagus, the pressure being highest at the upper limit of the sphincter. During swallowing, the tone of the inferior pharyngeal constrictor and cricopharyngeus is reduced at the same time as the group of muscles that elevate the hyoid cartilage and associated structures contract (Cook et al. 1989). The latter action causes the larynx to be pulled anteriorly and upwards (Cook et al. 1989). At the same time, the attachments of the inferior pharyngeal constrictor and cricopharyngeus muscles to the larynx cause the upper end of oesophagus to be drawn upwards along with the larynx. However, the bulk of the oesophagus is prevented from being drawn forward and upwards due to its posterior wall being bound to the anterior surface of the neck vertebrae by fascia (Curtis 1982). As a result of the combined action of these three sets of muscles, the UOS opens as it is drawn upwards and envelops the bolus at the same time as the action of the tongue and contraction of the upper and middle pharyngeal constrictors raise the intrapharnygeal pressure and cause the bolus to be ejected from the oral cavity (Singh and Hamdy 2005) (Fig. 7.3). The anterior and upward movement of the larynx also causes the pressure to be lowered in the empty oesophageal lumen, which aids swallowing by sucking the bolus and any expressed fluid into the open oesophagus. Further, the continuing contraction of the upper and middle pharyngeal constrictors during these actions maintains high pressure in the laryngopharynx and prevents any backflow.

The action of the various muscular components that contribute to swallowing is governed by a pattern generator in the central nervous system that acts via the vagus, superior laryngeal and glossopharyngeal nerves (Mu and Sanders 1996). While the sequence in which muscles are activated during swallowing is preset, the speed and duration of activation of each set of components can be modulated according to the physical characteristics of the bolus (Dantas et al. 1990).

The transit time of a bolus through the pharynx is influenced by the viscosity of the bolus but not its volume (Dantas et al. 1990). Hence, the rate limiting factor appears to be the speed with which the bolus can be moulded as it enters and traverses the narrowing confines of the pharynx. However, the extent to which the larynx is elevated at the commencement of swallowing is influenced by bolus volume (Dantas et al. 1990). Thus the UOS is given time to rise further, and presumably gape wider, in order to receive a larger bolus. Both the volume and the viscosity of the bolus influence the duration of the relaxation phase of the UOS at the conclusion of swallowing. The period of relaxation becomes more prolonged as the time taken to mould larger and more viscous boluses during their entry into the oesophagus increases. Some of this moulding may result from the inherent elasticity of the upper

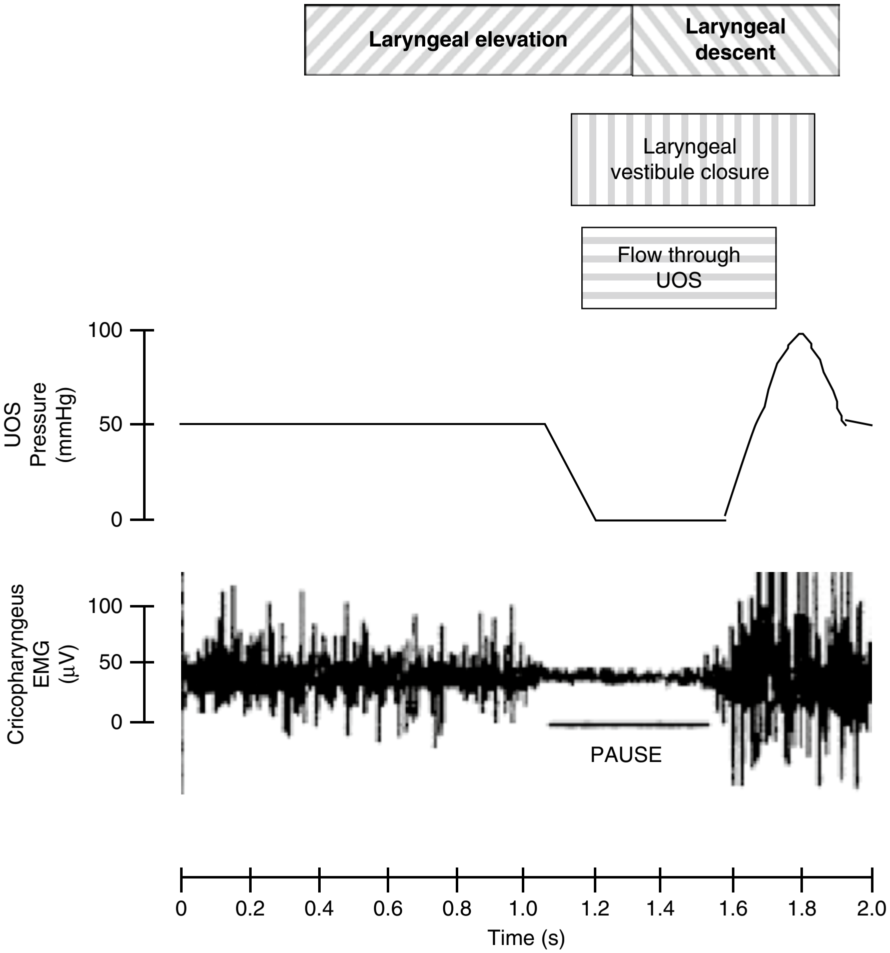


Fig. 7.3 Time line showing the order of contractile events, and changes in UOS pressure, during a normal swallow. (Singh and Hamdy 2005)

oesophageal opening owing to the significant amounts of elastic connective tissue in its walls (Bonington et al. 1988; Bonington et al. 1987). While this elasticity may increase the work of swallowing and the degree of friction of the walls with the bolus, the tight application of the oesophageal wall to the bolus will reduce the likelihood of any backflow of liquid expressed from the bolus.

Should the need arise; the contraction sequence of the upper oesophageal musculature during the operation of the sphincter can be altered so as to allow retrograde transit of material, i.e. regurgitation. In particular, the drop in intraluminal pressure that accompanies belching results from relaxation of the cricopharyngeus muscle (Monges et al. 1978), while the drop in intraluminal pressure that accompanies vomiting and retching results from the relaxation of both the cricopharyngeus and the inferior pharyngeal constrictors (Jacob et al. 1990; Kristmundsdottir et al. 1990).

7.3.2 The Lower Oesophageal Sphincter: Conditional Two-Way Travel

Reflux of gastric contents through the oesophagogastric junction is normally prevented by three components: a 2–3-cm-long thickening of the circular muscle layer in the walls of the distal oesophagus (the lower oesophageal sphincter); the right crus of the diaphragm, which surrounds the oesophagus at its point of transit from the chest; and the upper part of a sling of oblique gastric muscle which encircles the oesophagus just below the point of its transit into the abdomen. The muscular activity of these elements is inconstant. Elements of the vagus nerve that supply the lower oesophageal sphincter cause both tone and phasic activity to increase in synchrony with gastric phase III MMCs, while the periodic increase in the muscular tone of the right crus with inspiration exerts pressure on the oesophagus causing the local intraoesophageal pressure to rise (Boeckxstaens 2005). Both of these mechanisms act in concert to prevent reflux in the face of fluctuating intra-abdominal, thoracic and intragastric pressure (Mittal and Balaban 1997).

The lower oesophageal sphincter relaxes during both swallowing and peristalsis in response to a pattern generator in the swallowing centre of the brain stem (Jean 2001) to allow transit of boluses to the stomach. This relaxation is coincident with relaxation of the upper sphincter and continues until the descending wave of peristalsis reaches it (Gregersen 2003). The lower oesophageal sphincter also relaxes for a short time during belching, an action mediated by the vagus and termed transient lower oesophageal relaxation (TSLOR) (Hirsch et al. 2002; Mittal and McCallum 1987).

7.3.3 The Pyloric Sphincter: Morphological and Physiological Adaptation with Dynamic Restriction of Onflow

The pyloric lumen is narrowed by the combined action of two slings of smooth muscle that encircle it: a smaller, less prominent, proximally situated, pyloric loop; and a more powerful distal pyloric loop. The combined action of these two muscular divisions draws the portion of the pylorus situated at the distal end of the greater curvature towards a pad of fat and connective tissue situated at the distal end of the lesser curvature, i.e. the pyloric torus (Ramkumar and Schulze 2005). Any remaining gap between the two structures is plugged by a distally prolapsing fold of mucosa (Biancani et al. 1980; Williams 1962).

The manner in which the action of the pyloric sphincter is coordinated with phasic and tonic activity in the stomach and duodenum has been the subject of some debate (Pallotta et al. 1998). There is extensive evidence that the occlusive action of the pyloric sphincter is under close neural, humoral (Cai and Gabella 1984; Daniel et al. 1994; Malbert et al. 1995; Ramkumar and Schulze 2005; Sarna 1996; Yuan et al. 2001) and mechanosensory (Lentle et al. 2010) control. However, morphological evidence suggests that there can be little neural or other reflexive coordination between contractile events in the distal antrum and proximal duodenum during periods when the sphincter is open (Cannon 1911). Hence there is a significant reduction in ICC-MY, the cellular components that generate and convey slow-wave potentials to smooth muscle cells, and a corresponding absence of slow waves around the pyloric sphincter (Wang et al. 2005). Further, there is a lengthwise discontinuity in the circular smooth muscle layer comprising a band of connective tissue that would interrupt any proximal-to-distal myogenic propagation of peristalsis (Cannon 1911). Taken together, these morphological features suggest that flow across the open pylorus may be governed solely by the hydrostatic conditions, i.e. the intraluminal pressure on either side of the sphincter, rather than by the coordinated progression of contractile activity across this area. Hence gastric contents would traverse the pylorus into the duodenum according to the pressure generated by gastric tone, i.e. the pylorus would modulate flow from a 'pressure pump' (Indireshkumar et al. 2000).

A body of evidence obtained during the postprandial period supports the existence of such a mechanism. Non-invasive ultrasound studies suggest that the pyloric sphincter remains patent for much of the time during the postprandial period, i.e. 41% of the first hour. During this period, a significant volume of fluid transits to the proximal duodenum at times when gastric pressure exceeds duodenal pressure (56% of events result in distad transpyloric flow) (Pallotta et al. 1998). Frequent 'to and fro' movements of chyme between the duodenal and antral lumen are also reported to occur during this period, i.e. 20% of all transpyloric flow events in the first hour after eating (Pallotta et al. 1998). In this case, duodenal distension resulting from the anterograde propulsion of duodenal contents is hypothesised to induce duodenal phasic activity (13% of events) (Pallotta et al. 1998), either via distension or shear stress (Wang et al. 2005), which then causes duodenal contents to be retropulsed into the pylorus (Hausken et al. 1992; King et al. 1984). Were such a hydrostatic mechanism to operate, then the frequency of retropulsive contractions would in part depend on resistance to onflow within the duodenum, and hence on the extent to which the duodenal brake is operating. It is possible that the type of duodenal motility is also regulated in relation to pyloric activity so that proximal duodenal distension results from a predominance of tonic or segmental activity in the more distal duodenum. Hence direct infusion of hydrochloric acid or of 5% sodium chloride into the duodenum of human volunteers was followed by tonic occlusion (Rao et al. 1996) which would decrease the likelihood of onflow and increase the likelihood of proximal duodenal distension and retropulsion. Such duodenal activity may also restrict the length of the small intestine that is perfused by digesta recently ejected through the pylorus, which may in turn control the rate of uptake of nutrients and the onset of nutrient-driven satiety (Meyer et al. 1998).

A subtle feature of the action of the pyloric sphincter during the postprandial period is its autonomy. Thus, during the postprandial period, transpyloric flow from antrum to duodenum is influenced by the proximity of antral contractions to the pylorus (Indireshkumar et al. 2000). The pyloric sphincter remains patent when antrocorporal contractions are traversing the proximal regions of the corpus and antrum, and is increasingly more likely to constrict as antral contractions approach the pylorus (Indireshkumar et al. 2000; Keinke et al. 1984). So that ultimately there is opposition between the increasingly efficient pumping activity of distally progressing antral contraction and the closure of the pylorus.

Work showing that pyloric closure in ex vivo (and hence vagotomised) stomachs is maintained for longer during ramped perfusion of viscous material than when watery material is perfused (Lentle et al. 2010) suggests that the development of high pressure in the region of the pylorus stimulates its autonomous closure. Thus during the postprandial period, the independent action of the pylorus allows flow from antrum to duodenum to occur only when the pressure differential across the pylorus is low. Such action may prevent larger particles from approaching and impacting in the pylorus (Brown et al. 1993). Further, the hydrodynamic effects of the forcible retropulsion of digesta from a closed pylorus may cause larger particles to be driven further proximally than finer ones, the mechanism acting as a ‘virtual impactor’ that clears larger particles from the pylorus and at the same time contributes to the sieving action of the gastric antrum (Lentle et al. 2010).

Conversely, a body of evidence indicates that during the interdigestive period, i.e. phases I, II and III of the MMC cycle, pyloric opening is integrated with contractile behaviour in the antrum. Thus gastric contents would traverse the pylorus into the duodenum when antral peristalsis reaches the pylorus, i.e. relaxation of the pylorus would then be integrated with the action of the antral ‘peristaltic pump’ (Indireshkumar et al. 2000) and not be autonomous. Such coordinated transpyloric flow occurs during all three phases of the MMC cycle (Savoye et al. 2003), and the opening of the pylorus and the transit of chyme to the duodenum is always associated with the termination of antrocorporal contractions at the duodenum (Savoye-Collet et al. 2003). These findings led to the hypothesis that during phase II, ‘transpyloric flow is determined by active coordinated processes involving not only antral contraction but also duodenal motility, and pyloric opening and closure’ (Savoye-Collet et al. 2003). As a result, MMCs can act as ‘housekeeping’ contractions that evacuate stomach contents by generating transpyloric flow that is modulated by the pyloric sphincter acting as an integrated ‘gatekeeper’ (Savoye et al. 2003).

A unifying hypothesis between these two apparently conflicting sets of findings is that the autonomy of the pylorus varies with the nature of the gastric contents (Pallotta et al. 1998; White et al. 1983). Thus pyloric activity becomes autonomous only when nutrient-rich meals are fed, otherwise, e.g. when non-nutrient material is present in the gastric lumen, pyloric opening occurs only in conjunction with antrocorporal contractions (Heddle et al. 1988; King et al. 1985; White et al. 1983). Such a nutrient-induced increase in pyloric autonomy is likely to be neurally mediated. This may explain why vagal denervation of the distal stomach accelerates the emptying of solid material (Mroz and Kelly 1977). Following the ingestion of a nutrient meal, vagal input may affect the motor activity in the proximal and distal stomach differently, thereby influencing their coordination, and hence the ‘autonomy’ of the pylorus, insofar as coordination of pyloric activity with antral contractions (Pallotta et al. 1998).

7.3.4 The Sphincter of Oddi: Junctional Inflow of Digestive Secretions from a Fibroelastic Reservoir

The system that modulates the flow of pancreatic and hepatic digestive secretions into the proximal small intestine is distinctive in a number of respects. It constitutes the only example in humans of an intestinal T-junction conformation by which a significant amount of fluid is added to the contents of the gut from a large reservoir. The walls of the ducts of the human hepatobiliary system are viscoelastic (Dodds 1990) and consist mainly of elastic tissue (Dodds et al. 1989), smooth muscle being limited to the wall of the gall bladder, the cystic (gallbladder) duct and the sphincter of Oddi (Dodds 1990). The ducts function as a low-pressure low-flow system (Dodds 1990) with pressure being determined by the rate of inflow from the liver, the contractile state of the walls of the gall bladder, and the state of constriction of the sphincter of Oddi.

7.3.4.1 The Morphology of the Sphincter of Oddi

In the majority of people, the common bile duct and pancreatic ducts fuse within the duodenal wall to form a common channel, the ampulla of Vater. There is no expansion of the two ducts at the point of their confluence, and hence the term ampulla is misleading. The sleeves of smooth muscle that are associated with the terminations of the biliary, pancreatic and ampullary ducts are distinct from the smooth muscle components of the duodenum and have been each regarded as functionally separate sphincters (the sphincter choledochus; the pancreatic sphincter; and the sphincter ampullae, respectively), the whole assemblage being termed the sphincter of Oddi. The sphincter that is closest to the lumen of the duodenum, i.e. the sphincter ampullae, comprises an inner layer of circular and an outer layer of longitudinal smooth muscle fibres which both continue to the tip of the common duct. The latter duct and associated musculature project as a papilla into the lumen of the duodenum. The muscular coat of the papilla, which is sometimes termed the sphincter papillae, can be recognised ultrasonographically (Wehrmann et al. 2001). In man, the array of ducts and sphincters traverse the wall of the duodenum obliquely, while in other species, the degree of obliquity and the morphology may differ (Boyden 1937; Toouli and Baker 1991). There is some variation in the morphology of the assemblage in man. In some people, an embryonic minor or accessory duodenal papilla persists and may help to reduce excessive pressure in the pancreatic duct (Ohta et al. 1991). The form of the projecting papilla varies and can be hemispherical, villus, cone or nipple shaped (Phillip et al. 1974). All forms have the sphincteric opening at the apex allowing bile to enter the duodenal lumen at some small distance from the wall.

The smooth muscle cells of the sphincter exhibit spontaneous myoelectrical activity which is modulated by ICC (Parr et al. 2003) and is closely correlated with the intestinal MMC cycle (Yokohata and Tanaka 2000). Thus during phase I of the MMC cycle, there is relatively infrequent myoelectric activity in the sphincter, while this activity increases in phase II, and is regular and strong during phase III (Yokohata and Tanaka 2000). The sensitivity of the sphincter to neuronal stimuli is governed by a number of hormonal agents, notably cholecystokinin (CCK) (Ryan and Cohen 1976). The secretion of CCK following the consumption of a meal, along with vagal override of the MMC cycle, promotes ongoing transition of bile into the duodenum (see the following section).

As noted earlier, there has been some debate as to whether the sphincter acts as a variable resistor with phasic and tonic radial constriction acting to restrict the outflow from a relatively pressurised biliary system, or whether the concerted action of longitudinal and circular muscles function as a peristaltic pump (Liu et al. 1992; Woods et al. 2005). Indeed, it appears that in some species the sphincter of Oddi can act as a pump at low intra-biliary pressure and a resistor at higher (>3.5 mm Hg) intra-biliary pressure (Grivell et al. 2004). In humans (Behar and Biancani 1987; Toouli et al. 1982), cats (Behar and Biancani 1980) and dogs (Lin and Spray 1969), the sphincter of Oddi acts principally as a site of variable resistance to flow, and hence the flow of bile from the ampulla into the duodenum is unlikely to be turbulent, given the low-pressure differential between the two sites. In species such as the guinea pig (Harrington et al. 1992), the American opossum (Hanyu et al. 1990) and the rabbit (Sarles et al. 1976), the sphincter of Oddi rhythmically propels fluid into the duodenum so that turbulence around the merging jet of bile may be increased. It is noteworthy that this seems to occur mainly in granivorous and herbivorous species in which proximal digesta is likely to contain higher proportions of indigestible residues and have correspondingly greater apparent viscosity than in carnivorous or omnivorous species.

While healthy human bile is thought to behave as a Newtonian fluid (Ooi et al. 2004) with somewhat higher viscosity than water, co-secretion of mucus, such as occurs when the ductal system is infected, may render it pseudoplastic (Gottschalk and Lochner 1990) and increase its apparent viscosity (Cowie and Sutor 1975).

7.3.4.2 The Morphology of the Hepatic Ductal System

The human hepatic ductal system comprises a branching system of intrahepatic ducts which conduct bile to the extrahepatic common bile duct. An accessory structure, the gall bladder, adjoins the common duct via the cystic duct at or near the point at which the common duct emerges from the liver.

The common bile duct has a greater volumetric capacity than the intrahepatic and cystic ducts (Duch et al. 1998). The walls of the common bile duct consist mainly of longitudinally oriented fibrous tissue and are more elastic than those of the hepatic and cystic ducts (Jian and Wang 1991). Thus, apart from conveying bile to the duodenal wall, the common bile duct forms an elastic conduit for the storage of bile.

The wall of the gall bladder comprises an inner mucosal layer, an intermediate muscular layer, and an outer connective tissue layer. The organisation of the smooth muscle in the wall of the gall bladder differs from that of the gut in that it comprises a randomly interwoven mesh of muscle bundles in a matrix of connective tissue rather than separate longitudinal and circular layers (MacPherson et al. 1984). This arrangement enables the spherical constriction of the lumen but does not allow for activities that depend on the separate action of the longitudinally or circumferentially oriented elements, such as are required for peristalsis.

The hormone CCK, secreted when food enters the duodenum, inhibits neurogenically mediated contraction of the sphincter of Oddi in human subjects allowing bile to exit from the ductal system into the duodenum (Geenen et al. 1980). CCK also influences the pressure–volume relationship within the lumen of the gallbladder to augment the flow of bile into the elastic ductal reservoir, in particular, the wall compliance decreases as the concentration of CCK increases (Ryan and Cohen 1976). This decrease in compliance results from an increase in the tone of the smooth muscle within the wall of the gallbladder (Mack and Todd 1968). Thus the secretion of CCK after the consumption of a fatty meal causes bile to be secreted into the duodenum and the gallbladder to empty in concert with gastric emptying (Dodds 1990), the rate of emptying of the gallbladder generally being maximal 45 min after consumption of the meal.

Bile is secreted continuously by the liver and accumulates in the elastic ductal system. There is no anatomic sphincter separating the gall bladder from the common bile duct (MacPherson et al. 1984), and hence bile also enters the gall bladder. The neuronal stimuli that promote closure of the sphincter of Oddi during the interdigestive interval also promote relaxation of the smooth muscle in the walls of the gall bladder increasing its compliance and allowing bile from the common bile duct to accumulate in the gall bladder (Mawe et al. 2006). Not all bile is secreted in association with a meal. It is also secreted intermittently during the interdigestive interval. This may result from increasing distension of the gall bladder, which reflexly decreases the basal tone, the lumen pressure, and the frequency of phasic contractions in the sphincter of Oddi, thereby allowing bile to flow into the duodenum (Thune et al. 1991). The gall bladder undergoes an alternating cycle of fill during the interdigestive interval becoming distended during the first half of each MMC cycle and emptying during the second half (Takahashi et al. 1986). This cyclic pattern of emptying has been hypothesised to prevent the formation of microcalculi (Dodds 1990) and to prevent bacterial overgrowth (Yokohata and Tanaka 2000).

The importance of a unidirectional flow of bile through the component sphincters is underscored by the fact that manometric studies of the sphincter that employ cannulation may engender pancreatitis (Steinberg 2003), and by studies showing that sphincter dysfunction is associated with the development of acute pancreatitis (Chen et al. 1998; Woods et al. 2005).

7.3.5 Ileocolic Sphincter: A Physiological Sphincter

The ileocolic region in mammals generally marks the point of transition between the sites of enzymatic and microbial digestion, and of a corresponding change from the rapid transit of more fluid digesta in a narrow bored tube to a more tardy transit of comparatively viscid digesta along a tube of larger diameter (Stevens and Hume 1995). In some species, the junction is marked by morphological specialisation, such as the sacculus rotundus in the rabbit (Snipes 1979). In others, such as man, there are no distinctive morphological features other than the simple abutment of the two segments. In humans, sphincteric control appears to result largely from physiological specialisation as is evidenced by the distinctive patterns of tonic contraction.

The human ileocolic sphincter (ICS) is said to lie just proximal to the anatomical junction of the ileum with the colon (Malbert 2005). Morphologically, it is not readily distinguishable from the rest of the ileum but is physiologically identifiable as an area of higher intraluminal pressure (Castell et al. 1970) that results from a local elevation of intrinsic tone and of phasic activity (Dinning et al. 1999). Ongoing elevation of contractile tone in the human ICS limits the onflow of digesta causing it to be retained in the terminal ileum, from where episodic phasic activity in the wall of the terminal ileum moves the accumulated digesta into the caecum. Thus particulate (Khosla et al. 1989; Wilding et al. 1991) and liquid (Spiller et al. 1987) phases of digesta have been shown to accumulate in the terminal ileum. Moreover, it has been suggested that temporal variation of the tone and contractile activity in the ICS may generate differential onflow of the retained solid and liquid phases of digesta (Hammer et al. 1993), the terminal ileum in effect acting as an ‘intestinal stomach’ (Hurst 1931).

There is evidence that distinctive patterns of motility in the terminal ileum, namely DCCs and PPCs (Quigley et al. 1984), control the emptying of material that has accumulated during periods of relative quiescence. However, as with the pyloric sphincter, there is little evidence of neuronal coordination of the action of the ‘physiological sphincter’ with contractile activity distal to the junction. The emptying of accumulated digesta into the caecum appears to lead to the onset of colonic contractions by hydrostatic rather than neurogenic stimulation (Dinning et al. 1999), a similar situation to that which is thought to occur in the coordination of the action of the antrum with that of the duodenum. Nevertheless, this hydrodynamic reflex is quite efficient in admixing incoming ileal with colonic digesta. Thus indium-labelled DPTA becomes dispersed throughout the human colonic lumen within 3 h of being injected directly into the caecal lumen (Krevsky et al. 1986).

The mode of action of this type of sphincter, with physiological rather than morphological specialisation, raises the question as to whether such specialisation exists at other sites, i.e. the modulation of flow by change in tonic or phasic contraction. A number of workers have suggested that the action of the ‘ileal brake’, a reduction of the transit rate of digesta in response to the entry of lipids into the small intestine (Spiller et al. 1984), results from a change in the pattern of motility from one of peristaltic propulsion to one of segmentation in the duodenum and proximal ileum (Huge et al. 1995). In the latter pattern, the sum of the pulsatile alternate segmentative narrowing of the intestinal lumen is thought to increase the hydrodynamic resistance to gastric outflow, thereby elevating gastric lumen pressure (Kwiatek et al. 2009). To date, no studies have reported whether any tonal changes in the radial dimension accompany the action of this brake, however, changes in duodenal diameter as well as the onset of phasic activity have been reported to influence gastric emptying (Keinke et al. 1984).

7.4 Control of Flow and Mixing of Intestinal Contents by Secretion and Absorption of Water

A final important means by which control is exerted over the physical process of digestion at a segmental level, albeit indirectly, is by the secretion or absorption of water. Thus the outcome of the balance between secretion and absorption in a particular gut segment will influence the relative proportion of solids in contained digesta, and hence influence its apparent viscosity and rheology. However, it is noteworthy that secretion and absorption may not promptly influence water activity, i.e. the proportion of free water that is available for enzymatic digestion, within the interstices of particulate matter. Hence in the stomach, the activity of digestive enzymes may be locally reduced where foods with generally low water activity have been consumed or where there are local high levels of readily soluble nutrients within matrices that undergo bulk erosion.

In the proximal segments of the gut, the volume of the three principal sites of secretion is sufficient to have a significant dilutional impact on the physical characteristics of digesta. Hence, the volume of human saliva is around 1.5 l/day, gastric secretions 2.5 l/day and bile 0.5 l/day. The volume of small and large intestinal secretions are less well defined in view of the fact that these segment have high absorption rates (6.5 l/day and 1.3 l/day, respectively) (Zhang 1996). It is not known whether the rheological characteristics of digesta directly influence the secretion of water at any of the three principal proximal sites of secretion. However, their rates of secretion would be sufficient to bring about substantial changes in digesta characteristics within a short period of time, given the volumes of the compartments in which these secretions operate. When appropriately stimulated, human salivary glands can secrete saliva at up to 50 ml/min/100 g tissue (Ma and Verkman 1999), the gastric juice mucosa at up to rate of 0.7 ml/min/100 g tissue (Granger et al. 1983), and the pancreas at 4.5 ml/min (Argent and Case 1994). Some of these secretory mechanisms appear to involve aquaporins, which allow the passive passage of water via the central channels of transmembrane protein tetramers (Shi et al. 1994) each tetramer being formed from small proteins of around 30 kDa (Ma and Verkman 1999). The direction of movement of water through such channels is determined by the osmotic gradient (Ma and Verkman 1999). Hence the rate of movement of water into the intestinal lumen is likely to be highest when the lumen content is at its greatest osmolarity, i.e. after a meal. Aquaporins are expressed at all three sites of water secretion, as well as at other sites in the intestinal mucosa from the oesophagus to the colon (Koyama et al. 1999). More specifically, aquaporins 1 and 8 are involved in pancreatic secretion, and aquaporins 1, 5 and 8 with salivary secretion (Matsuzaki et al. 2004). A number of intestinal secretory systems are known to augment the osmotic gradient in the lumen, e.g. the secretion of sodium ions by the glandular acini of the salivary gland (Nauntofte 1992) and the secretion of bicarbonate by the ductal cells of the pancreas (Argent and Case 1994). These systems may promote aquaporin-mediated movement of water into the lumen of the glands. However, with the exception of salivary secretion, experiments with aquaporin knockout mice suggest that most of the fluid transfer in the gastrointestinal tract cannot be explained solely by aquaporins (Matsuzaki et al. 2004).

It is likely that the fraction of water transported by aquaporins varies with local conditions within the lumen. Hence there is evidence that the density of transmembrane aquaporin molecules on the apical and other surfaces of the cell may be hormonally regulated, e.g. in the intrahepatic bile duct (Marinelli et al. 1997; Marinelli et al. 1999) and in the collecting duct of the kidney (Nielsen et al. 1995). However, there is ongoing debate (Ma and Verkman 1999) as to the relative contribution of aquaporin channels, paracellular movement (Soergel et al. 1968) and co-transport via the apical sodium-glucose co-transporter system (Meinild et al. 1998) to the absorption of water by the small intestine. For instance, up to 5 l/day can be absorbed across the brush borders of enterocytes via the intestinal glucose co-transporter SGLT1 alone (Loo et al. 1996).

The mechanism by which the colonic mucosa absorbs water does not appear to involve the operation of aquaporins but results from active ion transport by colonocytes within the colonic crypts into the interstitial fluid. The osmotic pressure of colonic contents is high and the epithelial junctions are tight (Ma and Verkman 1999), presumably to prevent osmotically induced paracellular movement of water into the lumen. Current evidence suggests that solutes are actively transported from the crypt lumen across a water impermeable barrier generating a hypertonic interstitial fluid with an osmotic pressure that exceeds that of the faeces which then draws more water from the lumen (Pedley and Naftalin 1993). Aquaporins (AQP4 and AQP8) do not appear to be present in cryptal colonocytes but may be present in the basolateral or subapical membranes of surface colonocytes (Calamita et al. 2001; Wang et al. 2000).

References

Andrews PL, Grundy D, Scratcherd T (1980) Vagal afferent discharge from mechanoreceptors in different regions of the ferret stomach. J Physiol 298:513-524

Anuras S, Cooke AR, Christensen J (1974) An inhibitory innervation at the gastroduodenal junction. J Clin Invest 54:529-535

Argent B, Case R (1994) Pancreatic ducts: Cellular mechanism and control of bicarbonate secretion. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Raven, New York, pp 1473–1497

Barlow JD, Gregersen H, Thompson DG (2002) Identification of the biomechanical factors associated with the perception of distension in the human esophagus. Am J Physiol 282:G683-689

Barraya L, Pujol Soler R, Yvergneaux JP (1971) La région oddienne: Anatomie millimétrique. Presse Med 79:2527-2534

Behar J, Biancani P (1980) Effect of cholecystokinin and the octapeptide of cholecystokinin on the feline sphincter of Oddi and gallbladder. Mechanisms of action. J Clin Invest 66:1231-1239

Behar J, Biancani P (1987) Pharmacologic characterization of excitatory and inhibitory cholecystokinin receptors of the cat gallbladder and sphincter of Oddi. Gastroenterology 92:764-770

Biancani P, Zabinski MP, Kerstein MD, Behar J (1980) Mechanical characteristics of the cat pylorus. Gastroenterology 78:301-309

Boeckxstaens GE (2005) The lower oesophageal sphincter. Neurogastroenterol Mot 17:13-21

Bonington A, Mahon M, Whitmore I (1988) A histological and histochemical study of the cricopharyngeus muscle in man. J Anat 156:27-37

Bonington A, Whitmore I, Mahon M (1987) A histological and histochemical study of the cricopharyngeus muscle in the guinea-pig. J Anat 153:151-161

Bornstein JC, Furness JB, Kunze WAA, Bertrand PP (2002) Enteric reflexes that influence motility. In: Brookes SJH, Costa M (eds) Innervation of the gastrointestinal tract. Taylor & Francis, London

Bosch A, Pena LR (2007) The sphincter of Oddi. Dig Dis Sci 52:1211-1218

Boyden EA (1937) The sphincter of Oddi in man and certain representative mammals. Surgery 1:25-37

Brookes S, Costa M (2002) Innervation of the gastrointestinal tract. Taylor & Francis, London

Brookes SJH, Chen BN, Costa M, Humphreys CMS (1999) Initiation of peristalsis by circumferential stretch of flat sheets of guinea-pig ileum. J Physiol 516:525-538

Brown BP, Schulze-Delrieu K, Schrier JE, Abu-Yousef MM (1993) The configuration of the human gastroduodenal junction in the separate emptying of liquids and solids. Gastroenterology 105:433-440

Cai WQ, Gabella G (1984) Structure and innervation of the musculature at the gastroduodenal junction of the guinea-pig. J Anat 139:93-104

Calamita G, Mazzone A, Bizzoca A, Cavalier A, Cassano G, Thomas D, Svelto M (2001) Expression and immunolocalization of the aquaporin-8 water channel in rat gastrointestinal tract. Eur J Cell Biol 80:711-719

Cannon WB (1911) The mechanical factors of digestion. Edward Arnold, London

Castell DO, Cohen S, Harris LD (1970) Response of human ileocecal sphincter to gastrin. Am J Physiol 219:712-715

Chen JWC, Saccone GTP, Toouli J (1998) Sphincter of Oddi dysfunction and acute pancreatitis. Gut 43:305-308

Code CF, Schlegel JF (1973) The gastrointestinal interdigestive housekeeper: Motor correlates of the interdigestive myoelectric complex of the dog. Proceedings from the Fourth International Symposium on Gastro-Intestinal Motility. Mitchell Press, Vancouver, pp 631-634

Conklin JL, Christensen J (1975) Local specialization at ileocecal junction of the cat and opossum. Am J Physiol 228:1075-1081

Cook IJ, Dodds WJ, Dantas RO, Massey B, Kern MK, Lang IM, Brasseur JG, Hogan WJ (1989) Opening mechanisms of the human upper esophageal sphincter. Am J Physiol 257:G748-759

Costa M, Sanders KM, Schemann M, Smith TK, Cook IJ, de Giorgio R, Dent J, Grundy D, Shea-Donohue T, Tonini M, Brookes SJ (2005) A teaching module on cellular control of small intestinal motility. Neurogastroenterol Mot 17(Suppl. 3):4-19

Cowie AGA, Sutor DJ (1975) Viscosity and osmolality of abnormal biles. Digestion 13:312-315

Cuche G, Malbert CH (1998) Relationships between cecoileal reflux and ileal motor patterns in conscious pigs. Am J Physiol 274:G35-41

Curtis DJ (1982) Laryngeal dynamics. Crit Rev Diagn Imaging 18:29-80

Daniel E, Tougas G, Allescher HD, Vergara P, Fox-Threlkeld JA (1994) Mediators and enteric nerve pathways controlling gastric emptying. Dig Dis Sci 39:61-68

Dantas RO, Kern MK, Massey BT, Dodds WJ, Kahrilas PJ, Brasseur JG, Cook IJ, Lang IM (1990) Effect of swallowed bolus variables on oral and pharyngeal phases of swallowing. Am J Physiol 258:G675-681

Davison JS, Clarke GD (1988) Mechanical properties and sensitivity to CCK of vagal gastric slowly adapting mechanoreceptors. Am J Physiol 255:G55-61

Dellow D (1982) Studies on the nutrition of macropodine marsupials. 3. The flow of digesta through the stomach and intestine of macropodines and sheep. Aust J Zool 30:751–765

Dent J, Dodds WJ, Sekiguchi T, Hogan WJ, Arndorfer RC (1983) Interdigestive phasic contractions of the human lower esophageal sphincter. Gastroenterology 84:453-460

Dikeman C, Fahey G (2006) Viscosity as related to dietary fiber: A review. Crit Rev Food Sci Nutr 46:649-663

Dinning PG, Bampton PA, Kennedy ML, Kajimoto T, Lubowski DZ, De Carle DJ, Cook IJ (1999) Basal pressure patterns and reflexive motor responses in the human ileocolonic junction. Am J Physiol 276:G331-340

Dinning PG, Szczesniak MM, Cook IJ (2008) Determinants of postprandial flow across the human ileocaecal junction: A combined manometric and scintigraphic study. Neurogastroenterol Mot 20:1119-1126

Dodds WJ (1990) Biliary tract motility and its relationship to clinical disorders. Am J Roentgenol 155:247-258

Dodds WJ, Hogan WJ, Geenen JE (1989) Motility of the biliary system. In: Schultz SG (ed) Handbook of physiology the gastrointestinal system, pp 1055-1101

Dooley CP, Di Lorenzo C, Valenzuela JE (1992) Variability of migrating motor complex in humans. Dig Dis Sci 37:723-728

Duch BU, Petersen JAK, Gregersen H (1998) Luminal cross-sectional area and tension-strain relation of the porcine bile duct. Neurogastroenterol Mot 10:203-209

Ehrlein HJ, Ruoff G (1982) Cecal motility and flow of ingesta from the ileum to the cecum, appendix, and colon in rabbits. In: Wienbeck M (ed) Motility of the digestive tract. Raven New York, pp 475–481

Elliott TR, Barclay-Smith E (1904) Antiperistalsis and other muscular activities of the colon. J Physiol 31:272-304

Fackler K, Klein L, Hiltner A (1981) Polarizing light microscopy of intestine and its relationship to mechanical behaviour. J Microsc 124:305-311

Gao C, Arendt-Nielsen L, Liu W, Petersen P, Drewes AM, Gregersen H (2003) Sensory and biomechanical responses to ramp-controlled distension of the human duodenum. Am J Physiol 284:G461-471

Geenen JE, Hogan WJ, Dodds WJ, Stewart ET, Arndorfer RC (1980) Intraluminal pressure recording from the human sphincter of Oddi. Gastroenterology 78:317-324

Gottschalk M, Lochner A (1990) Behavior of postoperative viscosity of bile fluid from T-drainage. A contribution to cholelithogenesis. Gastroenterol J 50:65-67

Granger DN, Perry MA, Kvietys PR (1983) The microcirculation and fluid transport in digestive organs. Fed Proc 42:1667-1672

Gregersen H (2003) Biomechanics of the gastrointestinal tract: New perspectives in motility research and diagnostics. Springer Verlag, New York

Gregersen H, Drewes AM, Gilja OH (2005) Tension receptors: Theoretical construct or fact? Gastroenterology 128:803-804

Gregersen H, Hausken T, Yang J, Odegaard S, Gilja OH (2006) Mechanosensory properties in the human gastric antrum evaluated using B-mode ultrasonography during volume-controlled antral distension. Am J Physiol 290:G876-882

Gregersen H, Kassab G (1996) Biomechanics of the gastrointestinal tract. Neurogastroenterol Mot 8:277-297

Grivell MB, Woods CM, Grivell AR, Neild TO, Craig AG, Toouli J, Saccone GTP (2004) The possum sphincter of Oddi pumps or resists flow depending on common bile duct pressure: A multilumen manometry study. J Physiol 558:611-622

Hall KE, El-Sharkawy TY, Diamant NE (1986) Vagal control of canine postprandial upper gastrointestinal motility. Am J Physiol 250:G501-510

Hammer J, Camilleri M, Phillips SF, Aggarwal A, Haddad AM (1993) Does the ileocolonic junction differentiate between solids and liquids? Gut 34:222-226

Hanyu N, Dodds WJ, Layman RD, Hogan WJ, Chey WY, Takahashi I (1990) Mechanism of cholecystokinin-induced contraction of the opossum gallbladder. Gastroenterology 98:1299-1396

Harrington K, Bomzon A, Sharkey KA, Davison JS, Shaffer EA (1992) Differential sensitivities of the sphincter of Oddi and gallbladder to cholecystokinin in the guinea pig: Their role in trans-sphincteric bile flow. Can J Physiol Pharmacol 70:1336-1341

Hashmonai M, Go VL, Szurszewski JH (1987a) Effect of total sympathectomy and of decentralization on migrating complexes in dogs. Gastroenterology 92:978-986

Hashmonai M, Go VL, Yaksh T, Szurszewski JH (1987b) Effect of central administration of motilin on migrating complexes in the dog. Am J Physiol 252:G195-199

Hausken T, Ødegaard S, Matre K, Berstad A (1992) Antroduodenal motility and movements of luminal contents studied by duplex sonography. Gastroenterology 102:1583-1590

Heddle R, Fone D, Dent J, Horowitz M (1988) Stimulation of pyloric motility by intraduodenal dextrose in normal subjects. Br Med J 29:1349-1357

Heitz PU, Kasper M, Krey G, Polak JM, Pearse AG (1978) Immunoelectron cytochemical localization of motilin in human duodenal enterochromaffin cells. Gastroenterology 74:713-717

Hennig GW, Gregory S, Brookes SJH, Costa M (2010) Non-peristaltic patterns of motor activity in the guinea-pig proximal colon. Neurogastroenterol Mot 22:e207–217

Hipper K, Ehrlein HJ (2001) Motility of the large intestine and flow of digesta in pigs. Res Vet Sci 71:93-100

Hirsch DP, Tytgat GNJ, Boeckxstaens GEE (2002) Transient lower oesophageal sphincter relaxations-a pharmacological target for gastro-oesophageal reflux disease? Aliment Pharmacol Ther 16:17-26

Holloway RH, Blank EL, Takahashi I, Dodds WJ, Dent J, Sarna SK (1987) Electrical control activity of the lower esophageal sphincter in unanesthetized opossums. Am J Physiol 252:G511-521

Huge A, Weber E, Ehrlein H (1995) Effects of enteral feedback inhibition on motility, luminal flow, and absorption of nutrients in proximal gut of minipigs. Dig Dis Sci 40:1024-1034

Hukuhara T, Neya T (1968) The movements of the colon of rats and guinea pigs. Jpn J Physiol 18:551-562

Hurst AF (1931) Discussion on the function of the sympathetic nervous system. Proc R Soc Med 25:1597-1599

Husebye E (1999) The patterns of small bowel motility: Physiology and implications in organic disease and functional disorders. Neurogastroenterol Mot 11:141-162

Indireshkumar K, Brasseur JG, Faas H, Hebbard GS, Kunz P, Dent J, Feinle C, Li M, Boesiger P, Fried M (2000) Relative contributions of "pressure pump" and "peristaltic pump" to gastric emptying. Am J Physiol 278:G604-616

Itoh Z, Takeuchi S, Aizawa I, Mori K, Taminato T, Seino Y, Imura H, Yanaihara N (1978) Changes in plasma motilin concentration and gastrointestinal contractile activity in conscious dogs. Dig Dis Sci 23:929-935

Jacob P, Kahrilas PJ, Herzon G, McLaughlin B (1990) Determinants of upper esophageal sphincter pressure in dogs. Am J Physiol 259:G245-251

Jean A (2001) Brain stem control of swallowing: Neuronal network and cellular mechanisms. Physiol Rev 81:929-969

Jian C, Wang G (1991) Biomechanical study of the bile duct system outside the liver. Biomed Mater Eng 1:105-113

Johnson LR (2006) Physiology of the gastrointestinal tract. Academic Press, San Diego

Johnson PJ, Bornstein JC, Burcher E (1998) Roles of neuronal NK 1 and NK 3 receptors in synaptic transmission during motility reflexes in the guinea-pig ileum. Br J Pharmacol 124:1375-1384

Johnson PJ, Bornstein JC, Yuan SY, Furness JB (1996) Analysis of contributions of acetylcholine and tachykinins to neuro-neuronal transmission in motility reflexes in the guinea-pig ileum. Br J Pharmacol 118:973-983

Keinke O, Schemann M, Ehrlein HJ (1984) Mechanical factors regulating gastric emptying of viscous nutrient meals in dogs. Exp Physiol 69:781-795

Kellow JE, Borody TJ, Phillips SF, Tucker RL, Haddad AC (1986) Human interdigestive motility: Variations in patterns from esophagus to colon. Gastroenterology 91:386-395

Khosla R, Feely LC, Davis SS (1989) Gastrointestinal transit of non-disintegrating tablets in fed subjects. Int J Pharm 53:107-117

King PM, Adam RD, Pryde A, McDicken WN, Heading RC (1984) Relationships of human antroduodenal motility and transpyloric fluid movement: Non-invasive observations with real-time ultrasound. Br Med J 25:1384-1391

King PM, Heading RC, Pryde A (1985) Coordinated motor activity of the human gastroduodenal region. Dig Dis Sci 30:219-224

Kosterlitz HW, Lees GM (1964) Pharmacological analysis of intrinsic intestinal reflexes. Pharmacol Rev 16:301-339

Koyama Y, Yamamoto T, Tani T, Nihei K, Kondo D, Funaki H, Yaoita E, Kawasaki K, Sato N, Hatakeyama K (1999) Expression and localization of aquaporins in rat gastrointestinal tract. Am J Physiol 276:C621-627

Krevsky B, Malmud LS, D'ercole F, Maurer AH, Fisher RS (1986) Colonic transit scintigraphy. A physiologic approach to the quantitative measurement of colonic transit in humans. Gastroenterology 91:1102-1112

Kristmundsdottir F, Mahon M, Froes MMQ, Cumming WJK (1990) Histomorphometric and histopathological study of the human cricopharyngeus muscle: In health and in motor neuron disease. Neuropathol Appl Neurobiol 16:461-475

Kruis W, Azpiroz F, Phillips SF (1985) Contractile patterns and transit of fluid in canine terminal ileum. Am J Physiol 249:G264-270

Kunze WAA, Clerc N, Bertrand PP, Furness JB (1999) Contractile activity in intestinal muscle evokes action potential discharge in guinea-pig myenteric neurons. J Physiol 517:547-561

Kwiatek MA, Menne D, Steingoetter A, Goetze O, Forras-Kaufman Z, Kaufman E, Fruehauf H, Boesiger P, Fried M, Schwizer W (2009) Effect of meal volume and calorie load on postprandial gastric function and emptying: Studies under physiological conditions by combined fiber-optic pressure measurement and MRI. Am J Physiol 297:G894-901

Larson M, Schulze K (2002) Appearance of peristaltic reflex in isolated guinea pig ileum in response to boluses of air, water, oil, and cellulose. Dig Dis Sci 47:2644-2650

Lentle RG, Janssen PWM (2008) Physical characteristics of digesta and their influence on flow and mixing in the mammalian intestine: A review. J Comp Physiol B178:673-690

Lentle RG, Janssen PWM, Asvarujanon P, Chambers P, Stafford KJ, Hemar Y (2007) High definition mapping of circular and longitudinal motility in the terminal ileum of the brushtail possum *trichosurus vulpecula* with watery and viscous perfusates. J Comp Physiol B177:543-556

Lentle RG, Janssen PWM, Goh K, Chambers P, Hulls C (2010) Quantification of the effects of the volume and viscosity of gastric contents on antral and fundic activity in the rat stomach maintained *ex vivo*. Dig Dis Sci DOI 10.1007/s10620-010-1164-y:1-12

Lentle RG, Janssen PWM, Hume ID (2009) The roles of filtration and expression in the processing of digesta with high solid phase content. Comp Biochem Physiol A 154:1-9

Lin TM, Spray GF (1969) Effect of pentagastrin, cholecystokinin, caerulein, and glucagon on the choledochal resistance and bile flow of conscious dog. Gastroenterology 56:1178

Liu YF, Saccone GT, Thune A, Baker RA, Harvey JR, Toouli J (1992) Sphincter of Oddi regulates flow by acting as a variable resistor to flow. Am J Physiol 263:G683-689

Loo DDF, Zeuthen T, Chandy G, Wright EM (1996) Cotransport of water by the Na+/glucose cotransporter. Proc Natl Acad Sci USA 93:13367-13370

Ma T, Verkman AS (1999) Aquaporin water channels in gastrointestinal physiology. J Physiol 517:317-326

Mack AJ, Todd JK (1968) A study of human gall bladder muscle *in vitro*. Br Med J 9:546-549

MacPherson BR, Scott GW, Chansouria JPN, Fisher AWF (1984) The muscle layer of the canine gallbladder and cystic duct. Cells Tissues Organs 120:117-122

Malbert CH (2005) The ileocolonic sphincter. Neurogastroenterol Mot 17:41-49

Malbert CH, Mathis C, Laplace JP (1995) Vagal control of pyloric resistance. Am J Physiol 269:G558-569

Marik F, Code CF (1975) Control of the interdigestive myoelectric activity in dogs by the vagus nerves and pentagastrin. Gastroenterology 69:387-395

Marinelli RA, Pham L, Agre P, LaRusso NF (1997) Secretin promotes osmotic water transport in rat cholangiocytes by increasing aquaporin-1 water channels in plasma membrane. Evidence for a secretin-induced vesicular translocation of aquaporin-1. J Biol Chem 272:12984-12988

Marinelli RA, Tietz PS, Pham LD, Rueckert L, Agre P, LaRusso NF (1999) Secretin induces the apical insertion of aquaporin-1 water channels in rat cholangiocytes. Am J Physiol 276:G280-286

Matsuzaki T, Tajika Y, Ablimit A, Aoki T, Hagiwara H, Takata K (2004) Aquaporins in the digestive system. Med Electron Microsc 37:71-80

Mawe GM, Saccone GT, Pozo MJ (2006) Neural control of the gallbladder and sphincter of Oddi. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Academic Press, San Diego, pp 841–849

Meinild AK, Klaerke DA, Loo DDF, Wright EM, Zeuthen T (1998) The human Na+–glucose cotransporter is a molecular water pump. J Physiol 508:15-21

Mellander A, Jarbur K, Hemlin M, Sjovall H (2001) Effects of motility on epithelial transport in the human descending duodenum. Acta Physiol Scand 172:69-80

Meyer JH, Tabrizi Y, DiMaso N, Hlinka M, Raybould HE (1998) Length of intestinal contact on nutrient-driven satiety. Am J Physiol 275:R1308-1319

Mittal RK, Balaban DH (1997) The esophagogastric junction. N Engl J Med 336:924-932

Mittal RK, Liu J, Puckett JL, Bhalla V, Bhargava V, Tipnis N, Kassab G (2005) Sensory and motor function of the esophagus: Lessons from ultrasound imaging. Gastroenterology 128:487-497

Mittal RK, McCallum RW (1987) Characteristics of transient lower esophageal sphincter relaxation in humans. Am J Physiol 252:G636-641

Monges H, Salducci J, Naudy B (1978) The upper esophageal sphincter during vomiting, eructation, and distension of the cardia: An electromyographic study in the unanesthetized dog. In: Duthie HL (ed) Gastrointestinal motility in health and disease. MTP Press, Lancaster, pp 575–583

Mroz C, Kelly K (1977) The role of the extrinsic antral nerves in the regulation of gastric emptying. Surg Gynecol Obstet 145:369

Mu L, Sanders I (1996) The innervation of the human upper esophageal sphincter. Dysphagia 11:234-238

Nauntofte B (1992) Regulation of electrolyte and fluid secretion in salivary acinar cells. Am J Physiol 263:G823-837

Nielsen S, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA (1995) Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. Proc Natl Acad Sci USA 92:1013-1017

Ohta T, Nagakawa T, Kobayashi H, Kayahara M, Ueno K, Konishi I, Miyazaki I (1991) Histomorphological study on the minor duodenal papilla. J Gastroenterol 26:356-362

Ooi RC, Luo XY, Chin SB, Johnson AG, Bird NC (2004) The flow of bile in the human cystic duct. J Biomech 37:1913-1922

Orberg J, Baer E, Hiltner A (1983) Organization of collagen fibers in the intestine. Connect Tissue Res 11:285-297

Ozaki N, Sengupta JN, Gebhart GF (1999) Mechanosensitive properties of gastric vagal afferent fibers in the rat. J Neurophysiol 82:2210-2220

Pallotta N, Cicala M, Frandina C, Corazziari E (1998) Antro-pyloric contractile patterns and transpyloric flow after meal ingestion in humans. Am J Gastroenterol 93:2513-2522

Parr EJ, Kennedy AL, Mawe GM (2003) Lack of evidence for the existence of interstitial cells of Cajal in the gallbladder. Gastroenterology 124:A347

Pedley KC, Naftalin RJ (1993) Evidence from fluorescence microscopy and comparative studies that rat, ovine and bovine colonic crypts are absorptive. J Physiol 460:525-547

Penry DL, Jumars PA (1987) Modeling animal guts as chemical reactors. Am Nat 129:69-96

Phillip J, Koch H, Classen M (1974) Variations and anomalies of the papilla of vater, the pancreas and the biliary duct system. Endoscopy 6:70-77

Pluja L, Alberti E, Fernandez E, Mikkelsen HB, Thuneberg L, Jimenez M (2001) Evidence supporting presence of two pacemakers in rat colon. Am J Physiol 281:G255-266

Powell A, Bywater R (2001) Endogenous nitric oxide release modulates the direction and frequency of colonic migrating motor complexes in the isolated mouse colon. Neurogastroenterol Mot 13:221-228

Quigley EM, Phillips SF, Dent J (1984) Distinctive patterns of interdigestive motility at the canine ileocolonic junction. Gastroenterology 87:836-844

Ramkumar D, Schulze KS (2005) The pylorus. Neurogastroenterol Mot 17:22-30

Rao S, Lu C, Schulze-Delrieu K (1996) Duodenum as a immediate brake to gastric outflow: A videofluoroscopic and manometric assessment. Gastroenterology 110:740-747

Romański KW (2009) Migrating motor complex in biological sciences: Characterization, animal models and disturbances. Indian J Exp Biol 47:229-244

Ryan J, Cohen S (1976) Gallbladder pressure-volume response to gastrointestinal hormones. Am J Physiol 230:1461-1465

Sand J, Arvola P, Jantti V, Oja S, Singaram C, Baer G, Pasricha PJ, Nordback I (1997) The inhibitory role of nitric oxide in the control of porcine and human sphincter of Oddi activity. Br Med J 41:375-380

Sanders KM, Koh SD, Ward SM (2006) Organization and electrophysiology of interstitial cells of Cajal and smooth muscle cells in the gastrointestinal tract. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Elsevier, San Diego, pp 533–576

Sanders KM, Stevens R, Burke E, Ward SW (1990) Slow waves actively propagate at submucosal surface of circular layer in canine colon. Am J Physiol 259:G258-263

Sarles JC, Bidart JM, Devaux MA, Echinard C, Castagnini A (1976) Action of cholecystokinin and caerulein on the rabbit sphincter of Oddi. Digestion 14:415-423

Sarna S, Condon RE, Cowles V (1983) Enteric mechanisms of initiation of migrating myoelectric complexes in dogs. Gastroenterology 84:814-822

Sarna SK (1985) Cyclic motor activity; migrating motor complex: 1985. Gastroenterology 89:894-913

Sarna SK (1996) Nitronergic regulation of gastric motility and emptying. Curr Opin Gastroenterol 12:512-516

Sarna SK, Condon R, Cowles V (1984) Colonic migrating and nonmigrating motor complexes in dogs. Am J Physiol 246:G355-360

Sarna SK, Gleysteen JJ, Lang IM (1985) Is gastric cyclic motor activity a migrating motor complex. Gastroenterology 88:1570

Savoye-Collet C, Savoye G, Smout A (2003) Determinants of transpyloric fluid transport: A study using combined real-time ultrasound, manometry, and impedance recording. Am J Physiol 285:G1147-1152

Savoye G, Savoye-Collet C, Oors J, Smout A (2003) Interdigestive transpyloric fluid transport assessed by intraluminal impedance recording. Am J Physiol 284:G663-669

Schulze-Delrieu K (1991) Intrinsic differences in the filling responses of the guinea pig duodenum and ileum. J Lab Clin Med 117:44-50

Shi LB, Skach WR, Verkman AS (1994) Functional independence of monomeric chip28 water channels revealed by expression of wild-type mutant heterodimers. J Biol Chem 269:10417-10422

Singh S, Hamdy S (2005) The upper oesophageal sphincter. Neurogastroenterol Mot 17:3-12

Snipes RL (1979) Anatomy of the rabbit cecum. Anat Embryol (Berl) 155:57-80

Soergel K, Whalen G, Harris J (1968) Passive movement of water and sodium across the human small intestinal mucosa. J Appl Physiol 24:40-48

Song X, Chen BN, Zagorodnyuk VP, Lynn PA, Blackshaw LA, Grundy D, Brunsden AM, Costa M, Brookes SJH (2009) Identification of medium/high-threshold extrinsic mechanosensitive afferent nerves to the gastrointestinal tract. Gastroenterology 137:274-284

Spencer NJ, Smith CB, Smith TK (2001) Role of muscle tone in peristalsis in guinea-pig small intestine. J Physiol 530:295-306

Spencer NJ, Walsh M, Smith TK (1999) Does the guinea-pig ileum obey the 'law of the intestine'? J Physiol 517:889-898

Spiller RC, Brown ML, Phillips SF (1987) Emptying of the terminal ileum in intact humans. Influence of meal residue and ileal motility. Gastroenterology 92:724-729

Spiller RC, Trotman IF, Higgins BE, Ghatei MA, Grimble GK, Lee YC, Bloom SR, Misiewicz JJ, Silk DB (1984) The ileal brake-inhibition of jejunal motility after ileal fat perfusion in man. Br Med J 25:365-374

Steinberg WM (2003) Controversies in clinical pancreatology: Should the sphincter of Oddi be measured in patients with idiopathic recurrent acute pancreatitis, and should sphincterotomy be performed if the pressure is high? Pancreas 27:118-121

Stevens CE, Hume ID (1995) Comparative physiology of the vertebrate digestive system. Cambridge University Press, New York

Strege PR, Ou Y, Sha L, Rich A, Gibbons SJ, Szurszewski JH, Sarr MG, Farrugia G (2003) Sodium current in human intestinal interstitial cells of Cajal. Am J Physiol 285:G1111-1121

Tack J, Caenepeel P, Corsetti M, Janssens J (2004) Role of tension receptors in dyspeptic patients with hypersensitivity to gastric distention. Gastroenterology 127:1058-1066

Takahashi I, Kern MK, Dodds WJ, Hogan WJ, Sarna SK, Soergel KH, Itoh Z (1986) Contraction pattern of opossum gallbladder during fasting and after feeding. Am J Physiol 250:G227-235

Takeshima T (1971) Functional classification of the vagal afferent discharges in the dog's stomach. Nippon Heikatsukin Gakkai Zasshi 7:19-27

Thune A, Saccone GT, Scicchitano JP, Toouli J (1991) Distension of the gall bladder inhibits sphincter of Oddi motility in humans. Br Med J 32:690-693

Thuneberg L (1999) One hundred years of interstitial cells of Cajal. Microsc Res Tech 47:223-238

Timmermans JP (2001) Interstitial cells of Cajal: Is their role in gastrointestinal function in view of therapeutic perspectives underestimated or exaggerated? Folia Morphol 60:1-9

Toouli J, Baker RA (1991) Innervation of the sphincter of Oddi: Physiology and considerations of pharmacological intervention in biliary dyskinesia. Pharmacol Ther 49:269-281

Toouli J, Hogan WJ, Geenen JE, Dodds WJ, Arndorfer RC (1982) Action of cholecystokinin-octapeptide on sphincter of Oddi basal pressure and phasic wave activity in humans. Surgery 92:497-503

Wang KS, Ma T, Filiz F, Verkman AS, Bastidas JA (2000) Colon water transport in transgenic mice lacking aquaporin-4 water channels. Am J Physiol 279:G463-470

Wang XY, Lammers W, Bercik P, Huizinga JD (2005) Lack of pyloric interstitial cells of Cajal explains distinct peristaltic motor patterns in stomach and small intestine. Am J Physiol 289:G539-549

Wang ZY, Han YF, Huang X, Zhao P, Lu HL, Kim YC, Xu WX (2010) Pacemaking activity is regulated by membrane stretch via the CICR pathway in cultured interstitial cells of Cajal from murine intestine. J Biomech 43:2214-2220

Waterman SA, Tonini M, Costa M (1994) The role of ascending excitatory and descending inhibitory pathways in peristalsis in the isolated guinea-pig small intestine. J Physiol 481:223-232

Wehrmann T, Stergiou N, Riphaus A, Lembcke B (2001) Correlation between sphincter of Oddi manometry and intraductal ultrasound morphology in patients with suspected sphincter of Oddi dysfunction. Endoscopy 33:773-777

White CM, Poxon V, Alexander-Williams J (1983) Effects of nutrient liquids on human gastroduodenal motor activity. Br Med J 24:1109-1116

Wilding IR, Hardy JG, Maccari M, Ravelli V, Davis SS (1991) Scintigraphic and pharmacokinetic assessment of a multiparticulate sustained release formulation of diltiazem. Int J Pharm 76:133-143

Williams JA (1962) Closure of the pylorus. Br J Radiol 35:653-670

Wilmer A, Tack J, Coremans G, Janssens J, Peeters T, Vantrappen G (1993) 5-hydroxytryptamine-3 receptors are involved in the initiation of gastric phase-3 motor activity in humans. Gastroenterology 105:773-780

Wood JD (2006) Integrative functions of the enteric nervous system. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Academic Press, San Diego, pp 665–684

Woods CM, Mawe GM, Toouli J, Saccone GTP (2005) The sphincter of Oddi: Understanding its control and function. Neurogastroenterol Mot 17:31-40

Yokohata K, Tanaka M (2000) Cyclic motility of the sphincter of Oddi. J Hepatobiliary Pancreat Surg 7:178-182

Yuan SY, Costa M, Brookes SJH (2001) Neuronal control of the pyloric sphincter of the guinea-pig. Neurogastroenterol Mot 13:187-198

Zagorodnyuk VP, Chen BN, Brookes SJH (2001) Intraganglionic laminar endings are mechano-transduction sites of vagal tension receptors in the guinea-pig stomach. J Physiol 534:255-268

Zhang EB (1996) Intestinal water and electrolyte transport. In: Zhang EB, Sitrin MD, Black DB (eds) Gastrointestinal, hepatobilliary, and nutritional physiology. Lippincott-Raven, Philadelphia, pp 91–118

Zhao J, Liao D, Gregersen H (2005) Tension and stress in the rat and rabbit stomach are location and direction dependent. Neurogastroenterol Mot 17:388-398

Chapter 8
Local Motility, Flow and Mixing in Tubular Segments of the Gut

Contents

8.1 Introduction

Recent physiological thinking has moved away from a concept of distinctive types of contractile activity and towards the idea that contractile responses vary as a continuum in accordance with the sum of local and neuronal stimuli. In particular, they vary in the extent and direction of their propagation as well as in the degree of coordination between the circular and longitudinal muscle elements of the gut wall (Huizinga and Lammers 2009). It is nevertheless useful to examine the classes of contractile activity from a viewpoint of their efficacy in inducing mixing and propulsion, especially in respect of the form and function of the particular gut component in which they occur. For example, we can consider the particular features of oesophageal peristalsis to be specialised in expediently propelling a food bolus away from the larynx while minimising the backflow of any expressed fluid.

In this and the following chapter, we outline what is known regarding the form and function of contractile activity, particularly in relation to propulsive and mixing activities. While we pay due regard to activities in the nervous and other cellular components leading to the genesis and modulation of the various contractile forms and their function, we avoid detailed descriptions of their electrophysiological and pharmacological characteristics as these are well described elsewhere (Brookes and

R. G. Lentle, P. W. M. Janssen, *The Physical Processes of Digestion*,
DOI 10.1007/978-1-4419-9449-3_8,

Costa 2002). Propulsive and mixing activities are dealt with according to the shape of the gut components and the type of activity therein. In this chapter, we will deal with tubular segments of the gut that function simply to propel material from one section to another, or to mix and propel the contents. In the succeeding chapter, we will deal with segments that also function to store various elements of digesta.

8.2 Simple Propulsion

A number of segments of the intestinal tract function solely to transfer a discrete mass of food or digesta, i.e. a bolus, from one place to another. Two methods are found. The first occurs where contractions serve to reduce the volume of a proximal reservoir containing material that is the consistency of stiff paste causing it to flow or to undergo plastic deformation such that it is extruded into a smaller receiving segment. The second comprises propulsion of material by a wave of contractile activity that propels the bolus along the lumen with some deformation of the material from radial tension. In this section, we shall consider examples of each type in turn in the order of the segments in which they occur from proximal to distal.

8.2.1 Propulsion by Extrusion

Propulsion by extrusion occurs in the proximal and distal limits of the gastrointestinal tract. In both cases, movement of material contained in a proximally situated reservoir is secured by volume reduction causing contained material, which is generally of pasty consistency, to undergo plastic deformation. Volume reduction is secured by coordinated activity of smooth muscle in the walls of the proximal reservoir and of associated elements of striated muscle. The movements of these elements result from a combination of local neuronal propagation with a degree of volitional control and are governed by elements of the CNS and ENS in association with central pattern generators in the CNS (Jean and Dallaporta 2006). In both proximal and distal cases, voluntary control can be exerted over the commencement and the form of the subsequent activity, but once commenced, the activity is reflexly continued (Jean and Dallaporta 2006).

8.2.1.1 Deglutition: Transit from the Oral Cavity to the Pharynx and Oesophagus

The transit of ingested masses of solid food from the mouth to the oesophagus is complicated by the merging of the alimentary and respiratory tracts, the pharynx acting to convey either air to the larynx or food to the oesophagus. Hence, although the opening to the larynx is guarded by a keel-shaped flap, the epiglottis, solid material must be handled during deglutition in such a manner that solid residues do not lodge in the pharynx and subsequently enter the airway.

The process of deglutition can be viewed as comprising two stages, the formation and transfer of a food bolus across the pharynx and its passage from the laryngopharynx into the oesophagus via the upper oesophageal sphincter. The first stage comprises formation and extrusion of a bolus from the oral cavity to the narrower confines of the laryngopharynx, the second from the laryngopharynx to the upper oesophageal lumen. The latter process, though closely coordinated with the former, results in part from the action of the upper oesophageal sphincter.

Newly acquired pieces of food undergo fragmentation by mastication. Concurrent oral processing serves to agglomerate the fragments by admixture with salivary secretions to form a 'swallow-safe' bolus (Prinz and Lucas 1997), which will not fragment during deglutition. Fragmentation of the bolus can be prevented by a number of strategies that include: maximising the forces of cohesion between constituent food particles, which is achieved by reducing them to an optimum size and admixing them with saliva (Prinz and Lucas 1997); and reducing shear stress from friction of the bolus surface with the walls of the pharynx during transit, which is achieved by minimising the roughness of the bolus surface (Lillford 1991) and lubricating with mucins (Hutchings and Lillford 1988). It is noteworthy that the cohesion and reduction of surface friction must be sufficient to prevent fragmentation of the bolus during the significant degree of plastic deformation that must occur in the narrowing contours of the laryngopharynx and in the upper oesophageal sphincter.

Mechanics of Bolus Formation and Deglutition

The process of breaking down solid particles, i.e. chewing, and the formation of a bolus are governed by a central pattern generator (Lund 1991; Lund and Kolta 2006), which coordinates the movements of the jaw and tongue. Between the occlusive phases of each chewing cycle, the tongue catches masticated food that has been ejected from the molar dentary, mixes it with saliva, and presses the mixture against the hard palate before throwing it back into the molar dentary to undergo a further chewing cycle (Lucas 2004). Little is known of the manner in which masticated and ensalivated food is progressively accumulated on the tongue to form a mass of suitable size and with suitable physical characteristics for swallowing.

Once the swallowing reflex is initiated, the material in the bolus is extruded from the anterior (mesial) oral cavity into the oropharynx. The tongue is elevated by contraction of the striated muscles in the floor of the mouth. Concurrent with this elevation, coordinated constriction of the intrinsic striated muscles of the tongue causes it to approach the palate progressively from the front to the back of the mouth. The extruded material is then transported from the oropharynx to the oesophageal opening by progressive rearward projection of the tongue along with sequential contraction of superior, middle and inferior pharyngeal constrictor muscles. The latter movement is aided by the 'pharyngeal pump' generating a pressure gradient from the pharynx to the oesophagus (see below) (Ferguson et al. 1999).

Synchronous with contraction of the pharyngeal musculature, the larynx is elevated and the epiglottis descends to obturate its orad opening. The process of deglutition is rapid, taking around 0.7 s from initiation to completion (closure of the upper oesophageal sphincter). Concurrent with the final stage of the process and with the elevation of the larynx, the upper oesophageal sphincter relaxes and is drawn open between its posterior attachments to the anterior limits of the neck vertebrae and its anterior attachments to the cartilages of the larynx. This is discussed in the later section on oesophageal motility.

The above description of the mechanics of swallowing provides an overview of what is the outcome of a complex interplay of several reflexes. There are at least nine reflex mechanisms associated with the closure of the upper oesophageal sphincter, the closing of the airway, and the clearing of the oesophagus of gas liquids or solids (Shaker et al. 1993). These reflexes include a pharyngo-upper oesophageal constriction reflex, a pharyngoglottal adduction reflex, a pharyngeal reflexive swallow, a laryngo-upper oesophageal sphincter reflex, a laryngeal adductor reflex, an oesophagoglottal closure reflex, oesophago-upper oesophageal sphincter closure and opening reflexes, and a secondary oesophageal peristaltic reflex (Shaker et al. 1993). Hence it is likely that bolus material may initiate a series of reflexes by providing stimuli at a series of sites, and that these stimuli are based on different mechanical characteristics.

Effects of the Physical Properties of Food

A body of evidence indicates that the processes of mastication, bolus formation, and deglutition are modulated by the physical characteristics of the ingested food. Hence the duration of chewing and number of chewing cycles vary with the type of food that is consumed (Hiiemae et al. 1996). A number of oral sensory receptors are thought to provide information regarding the plasticity, cohesiveness and surface texture, i.e. slipperiness, (Seo et al. 2007) of the forming bolus. The physical characteristics of food particles may be directly assessed during mastication by sensory receptors in the dentine of the teeth (Paphangkorakit and Osborn 2000) and in the associated periodontal ligament (Linden 1990). The forces required to comminute and mix the bolus may be detected by receptors in the capsule of the jaw joint (Klineberg 1980) and in the associated musculature (Lucas 2004). Other sets of receptors, probably in the tongue, may detect flow within the bolus during the period when it is pressed against the palate (Dixon 1963; Lucas 2004; Palmer 1998).

This diverse sensory information, when collated, allows appropriate adjustment of the rates of passage of food boluses through the pharynx according to their physical properties (Okada et al. 2007; Saitoh et al. 2007). Thus watery material transits faster than viscous material, the latter requiring greater deformation (Dantas et al. 1990) to conform to the changing contours of the pharynx and palate. The swallowing of liquid material occurs in four sequential stages, i.e. oral preparatory, oral propulsive, pharyngeal and oesophageal. These generally occur in succession (Bosma 1957; Dantas et al. 1990; Dodds et al. 1990), although there is occasional leakage into the pharynx before the onset of deglutition (Linden et al. 1989; Robbins et al. 1992). When solid food is consumed, a large bolus may accumulate in the oropharynx prior to the commencement of the propulsive stage (Palmer 1998; Palmer et al. 1992). The work to be performed during bolus extrusion through the pharynx increases as solid foods accumulate in the oropharynx. The speed of progression of the front end of a bolus of semi-liquid material that is perceived as ‘soft’ is faster than one of a semi-liquid material that is perceived as ‘hard’ (Takahashi et al. 2002). Indeed, it has been suggested that the speed of pharyngeal transit of water (around 0.5 m/s) (Hasegawa et al. 2005) is sufficient to generate turbulence and backflow within the pharynx (Meng et al. 2005). This behaviour suggests that bolus propulsion is driven principally by the progressive anteroposterior squeezing action of the tongue against the palate (Nicosia and Robbins 2001). Viscous liquids would require greater applied force and consequently be driven into the pharynx more slowly.

Given the wide variety of physical properties exhibited by the different food items that are utilised by omnivores, such as man (Vincent 1990; Vincent et al. 1991), it seems likely that oral processing cannot simultaneously optimise all of the requisite characteristics of a bolus. For example, considerable mastication may be required to reduce the mean particle size of a particular food to a size that imparts the requisite surface smoothness, but the ongoing stimulation of the mucosa and teeth that stimulates ensalivation during these chewing cycles (Anderson et al. 1985) may render the bolus overly fluid (Lillford 1991; Prinz and Lucas 1997). It appears that, in man at least, such problems may be overcome by the adjustment of not only the processes of comminution and bolus formation, but also that of deglutition. Current research indicates that the latter results principally from differences in the sensory output during the initiation of swallowing rather than during transport of the bolus (Paterson 1999). Hence altering the size or consistency of the bolus has been shown to influence a number of processes that govern its subsequent transport to the oesophagus (Paterson 1999), namely retropulsion by the tongue (Miller and Watkin 1996), pharyngeal peristalsis (Shaker et al. 1993) and oesophageal opening (Cook et al. 1989).

Bolus Cohesion

Prinz and Lucas (1997) hypothesised that to obtain a ‘swallow-safe’ bolus, viscous cohesion within the bolus must be maximised so that it exceeds the surface tension forces that cause individual particles to adhere to the mucosa of the mouth and pharynx. Under such conditions, individual particles will be less likely to separate from the bolus and to inadvertently enter the airway following its reopening after swallowing. The force required to cause an individual particle to adhere to the mucosa (F_a) is given by

$$F_a = 4\pi r\lambda$$

where r is the radius of the particle and λ is the surface tension of the salivary film. The force of viscous cohesion between particles within the bolus (F_b) can be estimated from

$$F_b = \frac{3\pi\eta D^4}{64d^2t}$$

where η is the viscosity of the saliva, D is the radius of the bolus, d is the average distance between two particles within the bolus, and t is the time span over which particulate separation occurs. Hence the safest time to swallow would be when the net cohesive force *(F)* is maximal:

$$F = F_b - F_a$$

Plots of calculated F_b versus number of chews obtained using data from a subject chewing brazil nuts and carrots show that the net cohesive force increases to a maximum within 20–40 chews and subsequently declines. The latter decline fits in with observations that forced overchewing of food decreases F_b in the bolus from over hydration with saliva (Lucas 2004), i.e. by increasing *d.* While this model gives useful predictions for bolus formation in human subjects, it is noteworthy that the calculation of F_b does not allow for heterogeneity in the bolus, e.g. the effect of a thicker external coat of more viscous mucin, the effects of entanglement of particles (which would be significant in species that consume small food items with a high length to breadth ratio) or the effect of hydrophobicity of the surfaces of food items on cohesiveness (Lucas 2004).

Bolus Compliance

The passage of the bolus from the oral cavity into the oropharynx, laryngo-pharynx and upper oesophageal sphincter generally necessitates a change in its shape. The forces required to induce such change depend on the physical characteristics of the bolus. In particular, the forces may be required to overcome friction within a viscous liquid bolus, or to overcome viscous and elastic resistance within a viscoelastic bolus. In both cases, the situation is time dependent insofar as the deformation must take place within the time constraints of the swallowing process. As noted earlier, these constraints may vary to some extent according to the physical properties of the bolus but have a finite practical limit in that respiration is temporally suspended during swallowing. The outcome of this is that oral processing must be adjusted so that the physical characteristics of the bolus allow the time scale of the required deformation to lie within this limit. In effect, oral processing adjusts the ‘characteristic time’ of the material with respect to the (relatively invariable) characteristic time of the process, the ratio of these two quantities being termed the Deborah number (Steffe 1996), a dimensionless number that indicates the physical state of the material in regard to the time scale of the process to which it is subjected. Hence to quantify the efficiency of oral processing, the behaviour of swallow-safe bolus must be characterised, not only in terms of its apparent viscosity, but also in terms of its viscoelasticity and with due regard to the processing time of deglutition (Steele and Cichero 2008).

8.2.1.2 Extrusion at the Distal End of the Gastrointestinal Tract: The Rectoanal Junction

The mode of operation of the rectoanal junction has considerable similarity to that of the oral cavity and pharynx. The rectum comprises a reservoir where semisolid faecal material accumulates to be subsequently extruded into the anal canal during defecation. The anal canal is similar to the oesophagus in that it is relatively thick walled and that the lumen is almost completely occluded during times when no digesta is in transit. Hence the transit of faecal material is complicated by the need for the lumen to expand to accommodate it and for sufficient propulsive forces to develop in the rear of the faecal bolus to overcome any friction with the distended walls. As in the oesophagus, there is a need for conscious perception and control regarding the function of the anus so as to respond appropriately to the efflux of faecal material. Moreover, there is a corresponding incorporation of associated striated muscle, and linkage via spinal efferents and afferents to elements of the CNS.

The rectum comprises a region of expanded radial dimensions, which receives and stores distal colonic digesta pending its evacuation, and is located immediately distal to the sigmoid colon. When empty, the lumen of the receiving portion of the anal canal is reduced and the mucosa thrown into longitudinal folds, presumably as a result of a similarly high resting tone in the walls as occurs in the oesophagus (Gregersen 2003). The medial layer of the rectum comprises an inner layer of circular smooth muscle, which continues into the anal canal where it is termed the involuntary or internal sphincter, and a thinner outer layer of longitudinal smooth muscle, which similarly continues into the anal canal to be inserted ultimately into the perianal skin where it is termed the corrugator cutis ani. An additional layer of striated muscle envelops the media of the anal canal and is termed the external sphincter.

The manner in which the rectum empties, and how this is associated with the operation of the anal sphincters and the opening of the normally occluded anal canal has been subject to considerable controversy (Zbar et al. 2008) (Fig. 8.1–8.2). The operation of the rectum has been hypothesised to be governed by the action of a rectosigmoid pacemaker, which secures closure of the rectosigmoid junction prior

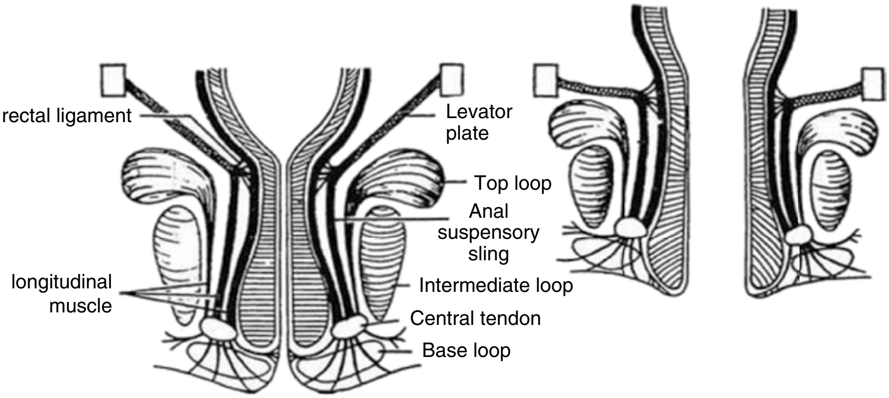


Fig. 8.1 Action of levator complex at rest *(left)* and during defecation *(right)* as hypothesised by Shafik (1980). Each levator ani is hypothesised to contract and shorten causing the walls of the anal canal to be drawn upwards and outwards. (Shafik 1980)

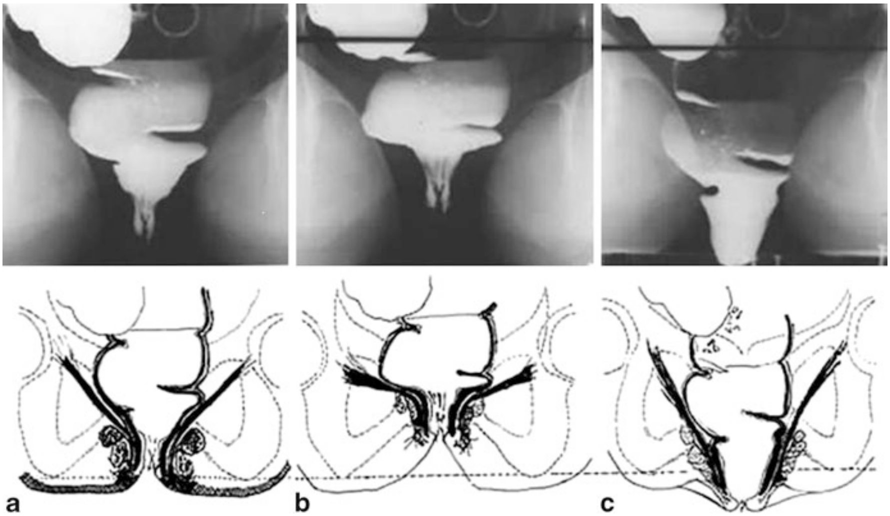


Fig. 8.2 Alternative hypothesis based on flattening of the contour of the levators ani. **a** The levators ani are funnel-shaped at rest. **b** Each levator ani ascends and becomes plate-shaped and the anus shuts tightly during squeezing. **c** During defecation, the levators ani descends (does not shorten and ascend) and becomes basin-shaped. (Li and Guo 2007)

to defecation and subsequently brings about an aborad progressing 'squeezing' contraction of the walls of the rectum. This causes a reduction in rectal volume, which together with anal opening allows faecal material to be expelled. On occasion, a number of such squeeze contractions are required to secure complete evacuation of the rectum. Sometimes, 'incomplete' squeezing contractions may occur against a closed anal sphincter (Shafik et al. 2003).

As noted above, the layers of smooth muscle in the anal canal are extensions of the circular and longitudinal layers of smooth muscle in the rectum. The distal regions of the inner circular layer are also associated with the distal ends of the more transversely oriented levators ani (Zbar et al. 2008), the proximal ends being attached to the walls of the pelvis. The levators ani comprise the overlapping sheets of pubococcygeus, iliococcygeus and puborectalis muscles. Of these, the puborectalis forms a loop that extends from the left inner surface of the symphysis pubis around the rectum to the corresponding point on the right. This is important for maintenance of faecal continence and normal defecation (Wood 2006). The complex mechanism by which this assemblage of muscles brings about opening of the anal canal and contributes to the evacuation of the rectum is not completely understood.

Tonic contraction of the internal anal sphincter (smooth muscle) and the puborectalis (skeletal muscle) normally blocks the passage of faeces into the anal canal. The entry of significant quantities of faeces into the rectum leads to its rapid filling and distension. Sensory mechanisms in the rectum detect this and activate the rectoanal reflex via the ENS. Elements of the ENS initiate the relaxation of the internal sphincter and activate spinal reflexes that cause the external sphincter to contract. This allows faeces to enter the anal canal, thereby stimulating sensory input to the brain (Wood 2006). Mechanoreceptors in the muscles of the pelvic floor detect changes in the orientation of the anorectum as faecal material enters this region. This results in a conscious sensation of rectal fullness (Wood 2006). However, it is the sensory receptors of the anal canal and surrounding skin that relay neuronal information to the spinal cord, which allow discrimination between the presences of either gas, liquid or solids in the anal canal.

The programmed sequence of contractile behaviour of smooth muscle during defecation includes an initial shortening of the longitudinal muscle coat of the sigmoid colon and rectum, followed by strong contraction of the circular muscle in these regions (Kamm et al. 1992). Recent work using CT defaecography suggests that the levators ani assume a plate-shaped form during the early phase of defecation, which aids in squeezing the rectum, while these same muscles assume a basin-shaped configuration during the opening of the anal canal and defecation (Li and Guo 2007) (Fig. 8.2). This change in orientation is thought to result from the levators acting as a fulcrum and pulling progressively to open the anal canal as they rotate downwards.

While the transit of faecal material through the rectum may be considered to be broadly analogous to the propulsion of a viscid pseudoplastic (power law) fluid through a tube (Patel et al. 1973), evacuation of the rectum appears to result primarily from pressure-induced plastic deformation of the contents rather than from peristalsis (Shafik et al. 2003), a situation that is broadly analogous to the final stage in the swallowing of a food bolus. In both cases, the plastic deformation required is minimised; in the former, by the action of the levators in drawing the walls of the rectum and anal canal outwards; in the latter, by the action of the pharyngeal and suprahyoid muscles drawing the upper end of the oesophagus upwards and outwards. Hence both faecal consistency and rectal tone may influence the ease of evacuation and transit through the open anal canal. Strain and tone in the distended rectum is difficult to determine given the complex geometry and effects of adjacent pelvic structures during distension (Frøkjær et al. 2006). Failing full rheological assessment along the lines suggested for food boluses, faecal consistency may be best performed using a back extrusion device rather than a rheometer (McRorie et al. 2000) as this mimics plastic deformation during defecation, i.e. the movement of a more ‘solid-like’ viscoelastic material that flows slowly in response to a propulsive force sustained over a relatively long period. However, due regard must be paid to the time characteristics that operate in the process of defecation.

The control of defecation is to some extent under conscious control in that voluntary inhibition of contractile squeezing and evacuation can render rectal contractions incomplete. There has been debate regarding the manner in which this occurs, one hypothesis being that rectal distension is accompanied by progressive tightening of the puborectalis muscle sling that surrounds the rectum (Shafik 1991). Later work suggested that this was accompanied by reflex tightening of the internal rectal sphincter (Shafik 1993).

8.2.2 Simple Propulsion: Oesophageal Contractile Activity

The oesophagus functions mainly to transfer ingested material, i.e. fluids and solid boluses, from the mouth to the remainder of the digestive tract. As such, its action provides insight into the simplest type of activity within a tubular section of the gut, i.e. propulsion, albeit with an element of extrinsic control by a central pattern generator (Jean and Dallaporta 2006).

8.2.2.1 Oesophageal Motility

The contractile activity of the oesophagus differs from that of the small intestine in a number of respects. The oesophageal lumen is almost completely occluded at times when no ingesta or digesta is in transit, and is reduced to an ellipsoid cavity of a few millimetres radius (Mittal et al. 2006). Moreover, there is considerable buckling of the inner mucosal layer within the resting oesophagus (Gregersen 2003). This seems likely to result from residual compressive strain as is evidenced by differences in the opening angles of the various muscular and mucosal layers of fresh transverse sections of the oesophagus (Gregersen et al. 1999). Hence occlusion of the lumen by mucosal buckling may aid the action of the lower oesophageal sphincter (Boeckxstaens 2005) in reducing the involuntary reflux of gastric or oesophageal contents. However, the level of lumen occlusion that is maintained distal to an advancing peristaltic wave appears insufficient to impede the progress of liquid ingesta, which may rapidly transit the lumen (Allen et al. 2003), overtake peristalsis, and pool in the distal oesophageal lumen pending the arrival of the peristaltic wave (Clouse and Diamant 2006). Manometric studies show the negative pressure in the thorax is transmitted through the wall of the transthoracic portion of the oesophagus to the lumen (−15 mm Hg on inspiration, +5 mm Hg on expiration) indicating that the bulk of radial compressive strain generated in the wall is accommodated in mucosal strain, i.e. buckling. It is noteworthy that oesophageal manometry is also able to detect this radial tension (see below) (Gregersen 2003) along with tension from the action of longitudinal muscles, the latter being thought to enhance the stiffness of the oesophageal wall in the longitudinal direction (Gregersen 2003), particularly the short intra-abdominal portion (Lu and Gregersen 2001).

The oesophageal transit of a bolus involves passive rather than active downstream distension of an initially small lumen secured by concerted contraction of circular and longitudinal muscle layers in the rear of the bolus (Mittal et al. 2006) with a force sufficient to overcome the elastic resistance of the walls and their friction with the bolus. Such dynamics differ from those in the small intestine in which propulsion through an initially larger lumen is secured by an advancing zone of partially occluding contraction with limited downstream dilatation (Lentle et al. 2007).

A further difference in the action of oesophageal and intestinal peristalsis results from the incorporation of striated muscle in the walls of the upper oesophagus and from the extrinsic control of motility via elements of the CNS (Clouse and Diamant

2006). Hence, in the upper portion of the oesophagus, where the wall contains striated muscle, propagation of peristalsis results solely from the sequential firing of a central pattern generator via successive vagal efferents (Jean 2001). In the remainder of the oesophagus, where the wall consists of smooth muscle, propagation may result from an integration of central and enteric neural mechanisms (Clouse and Diamant 2006). Such differences between gut segments in the muscular composition of the wall and in the neuronal organisation of motility, may account for the proximal to distal pattern of variation in the pressure within the oesophageal lumen during peristalsis (Clouse and Diamant 2006).

Simple models of oesophageal contraction have assumed that the oesophageal lumen constricts in a symmetric sinusoidal fashion (Misra and Pandey 2001) from simultaneous contraction of circular and longitudinal muscle layers in the rear of the bolus. In vivo M-mode ultrasound studies confirm that the thickness of the muscle layers increases in the region behind, i.e. aborad to, the bolus as does the intraluminal pressure (Mittal et al. 2005), and that the lumen becomes completely occluded at this site (Mittal et al. 2006) (Fig. 8.3). The same studies also show that the thickness of the oesophageal muscle layers is correspondingly reduced in the region where the lumen is distended by the bolus. This confirmed earlier work using combined B-mode ultrasonography and manometry, which showed that the overall

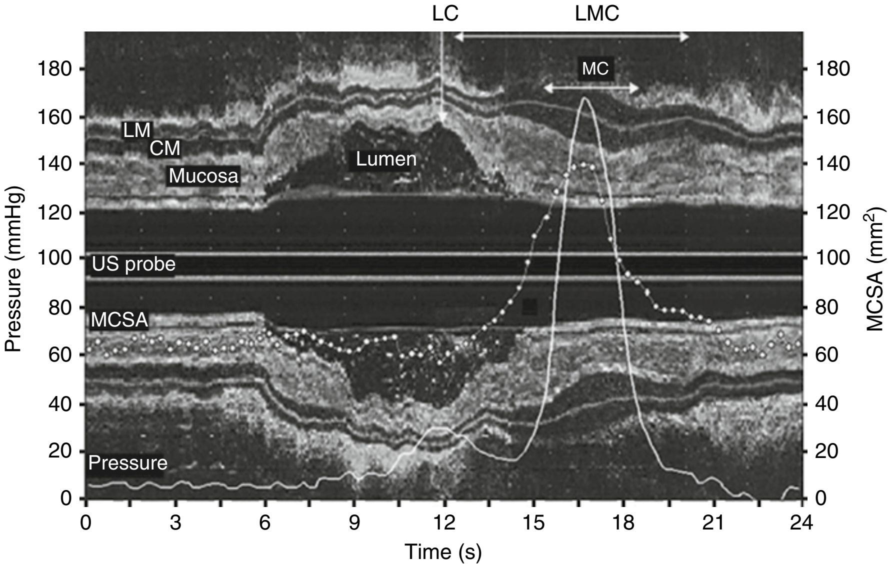


Fig. 8.3 Temporal coordination between manometric pressure and muscle cross-sectional area *(MCSA)* displayed on an M-mode US image. An M-mode image of the oesophagus at 5 cm above the LES during a swallow sequence is also shown. The onset and end of manometric contraction *(MC),* the onset and offset of longitudinal muscle contraction *(LMC),* and the onset of lumen collapse *(LC)* are indicated. Note that the onset of *LC* is closely associated with an increase in the *MCSA* and the duration of *MC* is significantly less than the duration of the increase in *MCSA*. (Mittal et al. 2006)

thickness of the muscular layers of the oesophagus was decreased, and that neither the circular nor the longitudinal muscle layer contracted in the region where the oesophageal lumen was distended (Nicosia et al. 2001). Further, it showed that the intensity of the contraction peaked in the two muscle layers at the same time (Mittal et al. 2006; Nicosia and Robbins 2001). However, the findings of the earlier study differed in that contraction in the rear of the bolus was reported to commence in the longitudinal layer before the circular layer (Nicosia et al. 2001). This difference is now thought to be due to a lag in the response time of the manometer (Mittal et al. 2006). The higher sensitivity of the M-mode scanning technique allowed changes in lumen contour to be assessed, which indicated that the commencement of circular muscle contraction was synchronous with that of longitudinal muscles (Mittal et al. 2006).

In summary, the bolus is propelled by synchronous contraction of longitudinal and circular muscle forcing it through a relaxed and locally expanded lumen. The synchronous local contraction of the longitudinal muscle is thought to increase the constrictive power of the circular muscle fibres and to reduce the stress in the wall by increasing its thickness (Nicosia et al. 2001; Sugarbaker et al. 1984a). Hence according to Laplace's law (stress = pressure × radius/wall thickness) an increase in wall thickness will reduce the effect of an increase in the radius of the lumen (Mittal et al. 2006). The effect of these forces, which act principally in the rear of the bolus, on the longitudinal contour of lumen dilation will vary according to the mechanical properties of the bolus rather than with the spatiotemporal characteristics of the propagating contraction and subsequent relaxation. However, it is conceivable that variation in the tone of the wall will influence the ease with which it is distended and the degree of its distension. At all events, it seems unlikely that the pattern of distension will be sinusoidal given that swallowed boluses generally are capable of undergoing deformation and thus are likely to undergo viscoelastic flow during transit, and that fluid boluses often flow ahead of the advancing zone of lumen constriction.

Intraluminal pressure during swallowing varies along the oesophagus rising to over 110 mm Hg (Clouse and Diamant 2006). Hence it is likely that the bolus undergoes a degree of compaction during transit. This, along with increased cohesion from interparticle bridging by viscous mucins, may account for recent evidence that oesophageal boluses remain intact for some time after entering the stomach (Marciani et al. 2001) allowing amylolytic digestion to proceed in situ (Gärtner 2001).

The level of coordination between longitudinal and circular muscle layers during peristalsis seems more consistent in the oesophagus than in the small intestine. Indeed, it has been proposed that a loss of coordination between longitudinal and circular muscle contraction may lead to a pathological local increase in intraluminal pressure and the formation of out-pouchings from the lumen (oesophageal diverticula). In addition, greater levels of control of longitudinal than circular oesophageal muscle contraction by the central rather than local systems (Sugarbaker et al. 1984b) may lead to the generation of dyssynergia when the distensibility of the wall of the oesophagus is locally compromised by fibrosis and scarring. This is not to say that isolated contraction of the longitudinal muscle layer of the oesophagus does not occur. Isolated longitudinal muscle contractions without a (radial contraction induced) rise in manometric pressure have been recorded in human subjects (Balaban et al. 1999; Nicosia et al. 2001). Animal studies indicate that in possums, cats and humans either isolated longitudinal contraction, or concerted radial and longitudinal contraction can be elicited according to the level of vagal stimulation (Dodds et al. 1978a; Dodds et al. 1978b; Sugarbaker et al. 1984a).

8.2.2.2 Variation of Oesophageal Peristalsis with Food Type

The physical characteristics of the food bolus are known to influence its speed of transit through the oesophagus (Frieling et al. 1996; Srinivasan et al. 2001; Wise et al. 2004). As noted earlier, watery material traverses the oesophagus more quickly than viscous material, due to it being retained in the proximal oesophagus for significantly less time (Wise et al. 2004), as well as transiting the oesophageal lumen more rapidly.

The extent to which the bolus stimulates the oral mucosa before and during the initiation of swallowing is known to influence the amplitude and speed of subsequent oesophageal peristalsis (Beyak et al. 1997), as well as the opening of the upper (Cook et al. 1989) and lower oesophageal sphincters (Hollis and Castell 1975). These effects may result either from afferent feedback from the mouth (Paterson 1999) influencing the action of the central pattern generator (Roman and Gonella 1987) or the direct effect of the physical characteristics of the material on the oesophageal wall and mucosa. In the latter respect, it is noteworthy that distension of the oesophageal lumen by a 'liquid bolus' (Dodds et al. 1973; Hollis and Castell 1975) causes the accompanying peristaltic wave to transit the oesophagus more slowly and with a greater amplitude and duration than peristalsis during a 'dry swallow'. However, a 2-ml liquid bolus has the same effect in decelerating oesophageal peristalsis as a 20-ml bolus (Hollis and Castell 1975). Hence it seems more likely that peristaltic velocity decreases once the lumen is distended above a certain threshold rather than in proportion to the degree with which local stretch receptors are stimulated.

8.2.2.3 Regional Variation in Oesophageal Peristalsis

As noted earlier, the dynamics of bolus transport also vary regionally, i.e. between the proximal, middle and distal segments of the oesophagus. The physical characteristics of the bolus, such as viscosity, variously influence aspects of motility in different segments (Wise et al. 2004). In addition, some of the variation between segments is likely to result from variations in the distensibility of the wall of the oesophagus with muscle type and with other structures associated with the serosal covering. In particular, distension may involve the stretching of collagen fibres in the outer layers of the oesophagus and those in para-oesophageal structures, such as the phreno-oesophageal ligament (Gregersen 2003; Gregersen et al. 1999).

Determination of the speed of transit of a bolus through the oesophagus is complicated by moulding, which reduces the diameter of the bolus as it traverses the oesophageal lumen. Given that the volume of the bolus is a constant, any decrease in its diameter will be accompanied by a proportionate increase in bolus length. Consequently, the difference between the speeds of transit of the leading and trailing edges of the bolus is increased in proportion to the rate at which it may be moulded. The lengthwise expansion of the bolus is likely to be greater at the leading edge as the bulk of contraction and reduction in wall compliance is at the rear of the bolus, especially when the material is easily moulded or flows readily. This has been confirmed in human subjects in whom the speed of progression of the head of a liquid bolus is faster than that of the body and tail (Nguyen et al. 1997). Thus three parameters are required to adequately determine the passage of a liquid bolus between an array of sensors on an oesophageal cannula: the speed of the bolus head (the time between the detection of the head of the bolus by the first sensor to its detection by the subsequent); the bolus presence time (the time between the detection of the head and the detection of the tail by a single sensor); and the bolus transit time (the time from the detection of the head of a bolus by one sensor to the time of detection of the tail of a bolus by the succeeding sensor). All these parameters are of longer duration in the distal than proximal oesophagus when either water or viscous materials are swallowed in either the lying or the upright position. However, the total bolus head transit time and the total bolus transit time between all the sensors are shorter for water than for viscous material (Wise et al. 2004). The amplitudes of the pressure waves in each oesophageal segment are lower for boluses of viscous fluid than those of water but vary between segments (Wise et al. 2004).

Food boluses are reported to traverse the proximal oesophagus more rapidly than the distal oesophagus (Wise et al. 2004). This may result either from differences in the pathways of neuronal activation or from the operation of the ‘pharyngeal pump’, a mechanism in which rearward movement of the tongue into the oropharynx generates a positive pressure on the proximal surface of the bolus while elevation of the larynx and opening of the upper oesophageal sphincter brings negative pressure to bear on the distal surface of the bolus (Buthpitiya et al. 1987).

8.3 The Small Intestine: Propulsion and Mixing

The principal mechanical functions of the walls of the small intestine are to secure appropriate propulsion of the contents and to mix them. It is important to note at the outset that shortening of the circular or longitudinal elements of the intestinal wall can alter local lumen volume, and hence displace the contents, i.e. induce propulsion, but that the speed with which intestinal smooth muscle contracts is generally not able to generate a sufficient rate of flow to bring about turbulent mixing, especially when the digesta is of a viscous nature. Hence it is likely that much of the mixing in the small intestine results from contractions that generate vortical flow (see Sect. 6.2) (Melville et al. 1975).

Primacy of the processes of propulsion and mixing varies, the propulsive requirement dominating at times when digestion is complete and no food has been recently ingested, the mixing requirement dominating during times when there must be admixture of ingested food with enzymes and subsequent absorption of nutrients. In Chap. 7, the manner in which control of the digestive system alternates according to the predominance of MMC cycling has been discussed. Thus, during the postprandial period, MMC cycling is absent or suppressed in the small intestine, although a range of differing contractile activities take place. Alternatively, contractile activities associated with MMCs take place at other times, their frequency varying with the phase of the cycle. However, our understanding is incomplete with regard to the form and frequency of the various types of contraction and how they change at various times and phases. Therefore, in this work, we detail what is known of the various types of contractile activity, albeit with an incomplete picture of the times at which they arise.

Three stereotypic forms of contractile activity are known to occur in the small intestine, notably segmentation (Cannon 1902, 1906, 1912), pendular contraction (Bayliss and Starling 1899), and peristalsis (Bayliss and Starling 1899). Segmentation and pendular movements result from localised contractions of circular and longitudinal muscles, respectively, and hence can only induce local flow. Therefore, it seems likely that these types of contraction serve to induce vortical mixing rather than net propulsion. Segmentation predominates when the lumen contains nutrient-rich material (Schemann and Ehrlein 1986). Rhythmic variation either in the length or the radial dimension of simple mechanical models (Fig. 8.4) (Macagno and Christensen 1981; Melville et al. 1975) is known to induce vortical flow.

Segmentation comprises a rhythmic stationary contraction in alternating segments along the long axis of the small intestine (Cannon 1902, 1906, 1912). It seems at first glance that it is well adapted to induce vortical mixing in a succession of longitudinally distributed cells along the long axis of the small intestine, perhaps corresponding to local neuronal domains, each of which induce alternating contraction and relaxation. However, considerations of fluid dynamics indicate that symmetrical contractions of the type indicated by Cannon are likely to induce

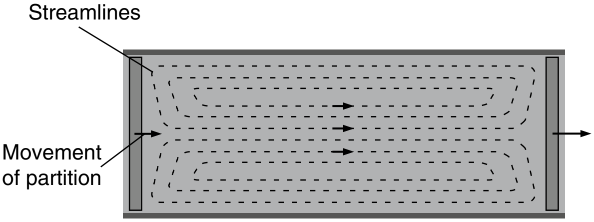


Fig. 8.4 Flow diagram in a cylindrical compartment based on that demonstrated in a mechanical model by Melville et al. (1975). Movement of the partitions relative to the cylindrical walls induces the formation of symmetrical vortices around the longitudinal axis

symmetrical vortical flow as indeed was shown in the mechanical flow model of Melville et al. (1975) (Fig. 8.4).

In such symmetrical vortical flow, any material within the plug of contained digesta will ultimately recirculate to the same position it occupied before displacement and intermixing with adjacent material will not occur. Hence vortical mixing can only occur if there is some form of asymmetry in the contractile process. In the case of pendular contractions, this is likely to result from the lengthwise propagation of slow waves that bring about longitudinal contraction in a limited segment of the cylindrical wall, generating an inherently asymmetrical pattern of contraction, which causes a length of intestine to assume a curved profile (Lammers 2005). In the case of segmentation, where propagation may be limited to a relatively small neuronal territory and be correspondingly more symmetrical, differences between the profiles of the intestinal segment during contraction and subsequent relaxation may engender asymmetry. One possible mechanism could be from variation in the localisation of residual strain within the intestinal wall and the extent of mucosal buckling (Gregersen 2003) with the degree of dilatation at the commencement and termination of each segmentation cycle.

Mixing may also occur during peristalsis. The amplitude of the contraction, i.e. the cross-sectional area of the lumen at the peak of peristalsis, influences the degree of mixing and the efficiency of propulsion. Modelling studies of non-occlusive peristaltic contractions (Jeffrey et al. 2003) indicate that vortices form during periods when the opposing walls of the intestine approach each other and when they move apart (see below). During peristaltic events that completely occlude the lumen, flow of digesta from the region of higher pressure formed in front of the advancing contraction to the region of lower pressure formed in the rear of the contraction is prevented, increasing the efficiency of propulsion while reducing the extent of vortex formation. Hence the extent of occlusion influences whether mixing or propulsion takes place. It is noteworthy that vortex formation during propulsive peristalsis is likely to be asymmetric as a consequence of its propagation, i.e. the distension is likely to be greater at the leading than the trailing edge of a propagating contraction due to a combination of displacement of the contents and advancement of the leading edge. The localisation of residual strain with respect to the mucosa and the extent of mucosal buckling (Gregersen 2003) will similarly vary with the degree of dilatation in the leading and following edges of the contraction. Additional mechanisms contributing to asymmetry include: phase differences between the circular muscle contraction at the oral and aboral ends of the occluded segment; and differences in responses of shear sensitive (pseudoplastic and non-Newtonian) digesta (Lentle et al. 2006; Lentle et al. 2005) to differing rates of muscle contraction and/or relaxation.

Vortical mixing may also be augmented by the grossly convoluted nature of the small intestinal lumen. Systems of twisted pipes are known to improve the residence time distributions (i.e. reduce the spread) of reactants and products in chemical reactors (Castelain et al. 1997). Similarly, twisted conformations are known to increase the rates of heat transfer from the walls of heat exchangers containing fluids with low Reynolds numbers (Mokrani et al. 1997). These increases in mixing are thought to be due to the induction of regular vortices termed Dean roll cells, which augment radial mixing, particularly when the angles between the bends are chaotic rather than in a helical sequence.

8.3.1 Peristaltic Activity

The term ‘peristalsis’ has been used to describe in vivo contractile events that propel digesta along the lumen of the intestine by totally or partially occluding it (Huizinga and Lammers 2009). The term ‘peristaltic reflex’ has been used to describe contractile events in ex vivo preparations that are induced by distension of the lumen (Bornstein et al. 2002). A degree of caution is required in generalising the results obtained from ex vivo preparations subjected to static increases in pressure with watery perfusates, such as physiological saline. This procedure will cause circumferential tension to increase along the entire length of the preparation generating a pattern of distension and mechanoreceptor stimulation that differs significantly from that obtained in vivo during the passage of a localised bolus of more viscid digesta. The latter pattern of distension is known to generate ascending excitatory electrical activity that triggers smooth muscle contraction proximal to the point of distension while at the same time generating inhibitory electrical activity distal to the point of distension (Costa et al. 2000), both types of activity propagating distally with the bolus. The former pattern of distension is thought to generate simultaneous excitatory and inhibitory activity along the length of the ex vivo preparation, with excitatory activity along the entire length and inhibitory activity being absent only in the most proximal region of the preparation. Thus the latter region constitutes the only site at which distension-induced contractile activity can commence (Bornstein et al. 2002), and propagation is generated by descending excitation progressively extinguishing inhibition. Consequently, the form of the peristaltic contraction may differ between the two situations due to the different spatiotemporal patterns of inhibition and excitation.

While the occlusive profile of bolus-driven peristalsis has not been mapped in detail, the profile of the peristaltic reflex has been mapped in isolated guinea pig small intestine distended with water (Fig. 8.5) (Schulze-Delrieu 1999). This profile departs markedly from the symmetric sinusoidal occlusion that was assumed in early computer models (Burns and Parkes 1967; Latham 1966). Peristaltic contractions progress longitudinally with the leading ‘shoulder’ propagating at a different rate to that of the trailing shoulder. As a result, the occluding segment (also called ‘coapting segment’) undergoes progressive lengthening and the contraction profile is generally axially asymmetric (Fig. 8.5–8.6). The profiles of the leading and trailing shoulders vary with the degree of volume loading, i.e. the amount of strain imposed. Consequently, the length of the occluding segment varies in proportion to the volume injected and is noticeably longer when the ends of the preparation are occluded. The author of this work did not state whether the partial occlusion of the lumen progressed to complete occlusion on any occasion. However, the results from other work using video image analysis of ex vivo preparations suggest that

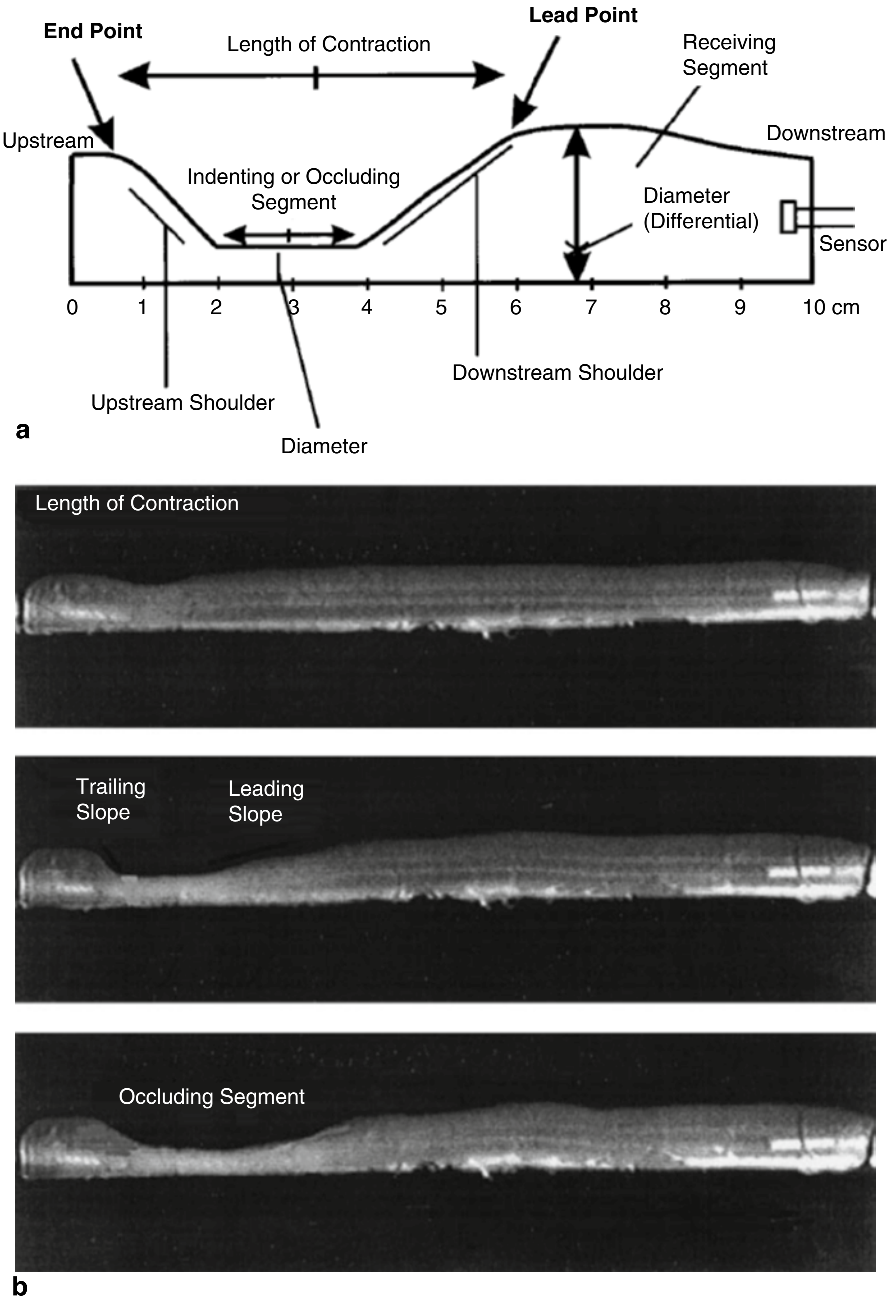


Fig. 8.5 Peristaltic contractile response following the injection of a 0.80-ml bolus into an open segment of guinea pig ileum. **a** Definition of visual parameters. The contraction indents mainly the antimesenteric wall. Within the occluding segment, mesenteric and antimesenteric walls run almost parallel to each other and intestinal diameter remains constant. **b** Peristaltic contraction in a

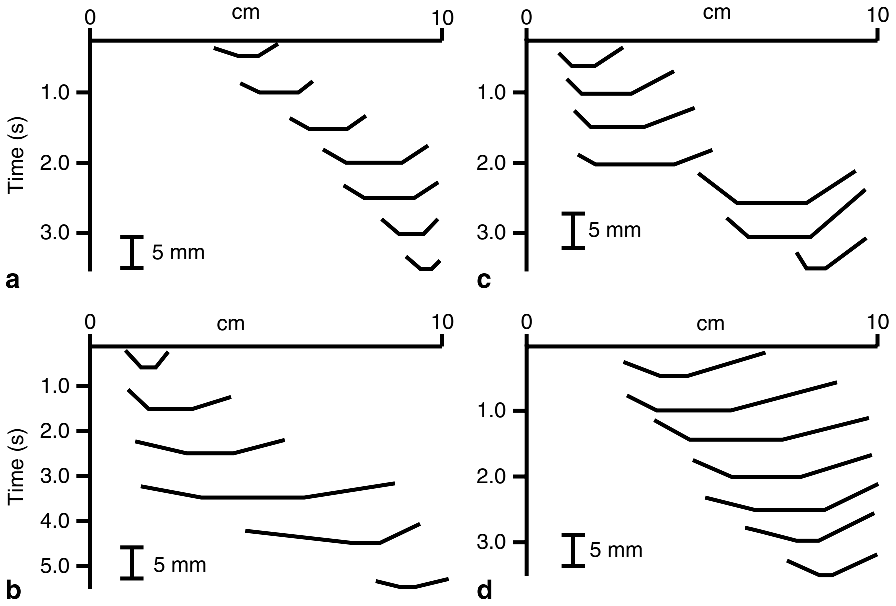


Fig. 8.6 Virtual configurations of contractions as a function of load conditions. Contractions were reconstructed from automated analysis of visual parameters. Their visual patterns are charted at specific points in time and at site of 10-cm segment at which they were then located. Each composite line is a proportional rendition of the length of the occluding segment and of the gradients and length of the shoulders. **a** Injection of 0.8-ml bolus into an open preparation. The occluding segment moves at a fairly steady rate from about 4–9.5 cm; its length increases steadily up to 2 s and then steadily decreases. **b** Injection of 1.6-ml bolus into an open preparation. This generates contraction of considerable length. Much of the length is made up of the shoulders. Shallow gradient of upstream shoulder indicates that post contraction the segment remains fairly collapsed. **c** Response to 0.8-ml bolus into closed segment. In first 2.0 s, the lead point advances as occluding segment lengthens. Between 2.0–2.5 s contraction suddenly shifts into distal half of preparation and deeply indents the lumen. This presumably reflects distension of both receiving and post contraction segment by displaced fluid. The length and gradient of the downstream shoulder then increase, indicating a bulging receiving segment. **d** Response to a 1.6-ml bolus delivered into a closed segment. Contraction moves over a shorter distance than when the preparation is open. (Schulze-Delrieu 1999)

10-cm segment viewed with the upstream end to left and its mesenteric border to bottom. The *top frame* was taken 1 s after bolus injection and the succeeding frames are 1 s apart. In the *top frame* an indentation develops along antimesenteric border. In the *middle frame,* the indentation deepens; the upstream shoulder is short and steep, the downstream shoulder long and shallow and the receiving segment downstream from contraction bulges slightly. In the *bottom frame,* the length of occluding segment has increased and the shoulders are nearly symmetric. (Schulze-Delrieu 1999) complete small intestinal occlusion may not occur in ex vivo preparations, the relative diameter on constriction being around 40% of that of the maximum diameter (Hennig et al. 1999; Lentle et al. 2007; Waterman et al. 1994). It is noteworthy that the amplitude of constriction is greater when the end of the ex vivo preparation is free to shorten (Hennig et al. 1999), and hence complete occlusion may occur in vivo where there are no limitations on the length.

Finite element matrix models based on the profiles shown in Fig. 8.6 indicated that pressures would be sufficiently elevated within the occluded segment to generate asymmetric vortical flow (Jeffrey et al. 2003). The extent and disposition of such vortical action around the peristaltic front were shown to depend upon the shape of the advancing peristaltic segment (Jeffrey et al. 2003). In particular, a contractile front with steep shoulders generated the larger wall pressures and affected a longer segment of gut than did those with flatter shoulders. The models also showed that the pressure pulse generated by the peristaltic contraction migrated from upstream to downstream of the occluded segment during peristaltic progression. Pressure differentials across the occluded segment generated reverse flow as was originally predicted by a mechanical model (Weinberg et al. 1971). This effect was more pronounced when peristalsis proceeded to complete occlusion over a long segment of gut.

The dynamics obtained in the computer simulation were recently confirmed by video recordings of an ink-marked bolus as it traversed an ex vivo preparation of guinea pig small intestine perfused from a reservoir with a standing pressure head (Schulze and Clark 2008). The recordings showed that peristaltic contractions cleared much of the fluid from the perfused segment but retrograde flow commenced on subsequent relaxation with vortical eddies forming around the upstream shoulders of the waning contraction. Mixing within the perfused segment was relatively inefficient given that the proximal end of the preparation was under a constant perfusion pressure and three or four peristaltic contractions were required to disperse the ink throughout the segment.

It is important to note that the video recordings (Fig. 8.5) were taken of ex vivo preparations in which the lengthwise patterns of strain distribution do not reflect those that occur in vivo, as discussed at the start of this section. Further, differences in the tone and distensibilities of the walls of different sections of the small intestine may complicate comparisons between them (Rao et al. 1996). Hence, distensibility may be sufficiently low to prevent generalised distension at a given hydrostatic pressure but still allow local distension, e.g. where greater resistance to outflow exists at the narrowed distal end of a preparation. Thus the site of onset and pattern of peristaltic contraction may vary between different segments of the intestine. Therefore, the differences in the site of onset of peristaltic contractions between duodenal and ileal segments reported in such preparations (Schulze and Clark 2008) may not reflect conditions in vivo. However, a recent study on a resected and perfused segment of rat jejunum with intact vascular connections showed that peristaltic behaviour was broadly similar to that obtained in the ex vivo Trendelenberg preparation (Bogeski et al. 2005).

Few studies have been conducted describing the form and characteristics of peristalsis in vivo or their effect on mixing. It is known that the frequency and amplitude of human duodenal contractions vary with pH and with fatty acid concentration of the contents (Gwynne et al. 2004; Rao et al. 1996). Similarly, the frequency, velocity of propagation, and width of contractile segment vary with nutrient content in the jejunum of the dog (Schemann and Ehrlein 1986). One study indicates that peristalsis is inefficient in promoting mixing in vivo (De Backer et al. 2008). These workers found that a bolus of florescent dye marker was dispersed over less than 20% of the total length after it had traversed the bulk of the small intestine.

Similarly, little work has been conducted to describe the transverse profile of the empty small intestinal lumen or the variation of this profile with intestinal tone. It is possible that the profile is of a flattened ellipsoidal configuration as in the oesophagus (Mittal et al. 2005). Recent LOC-I-GUT studies of human small intestinal profiles (Knutson et al. 2009) suggest that the profile of the small intestinal lumen during early distension may be somewhat ellipsoidal, although the authors do not comment on this or provide numerical data. This is important in regard to onflow of digesta given that it is theoretically possible for digesta to distend a flattened ellipse without bringing about any increase in wall tension.

Again, little is known of changes in transverse profile of small intestine during contractile activity. While the lumen radius appears to expand and constrict symmetrically during passage of a semisolid bolus through the oesophagus (Mittal et al. 2005), in the small intestine this may not always be the case. The lumen profiles of peristaltic contraction in segments of guinea pig ileum maintained ex vivo are reported to be asymmetric about the longitudinal axis, constriction occurring chiefly on the antimesenteric side of the ileum (Schulze-Delrieu 1999). It is not known whether this results from regional differences in the mechanical properties of the wall or from asymmetry of smooth muscle contraction.

8.3.1.1 The Structure of the Peristaltic Event

The peristaltic event may be viewed as having a structure comprising a zone of distension that travels in advance of a zone of constriction. The constricting zone comprises an upstream and downstream shoulder with an intervening uniformly occluding segment of variable length and degree of occlusion (Fig. 8.6). The configuration of the leading edge of a peristaltic contraction ultimately reflects the patterns of progression of circular and longitudinal muscle contractions within the small intestinal wall and their relative effects on the diameter of the lumen around the peristaltic event.

The Preceding Zone of Dilatation

A number of workers have reported that a zone of dilatation travels in advance of the zone of peristaltic constriction (Alvarez and Zimmermann 1927; Hennig et al. 1999;

Lentle et al. 2007). Such dilatation could result either from passive distension by lumen contents that have been expelled from and propelled in advance of the zone of peristaltic constriction, or from active distension caused by localised constriction of longitudinal muscles bringing about longitudinal shortening and width-wise expansion. High-fidelity spatiotemporal mapping of ileal peristaltic events showed that phasic radial expansion of the preceding zone occurred at the same time as radial constriction of the occluding segment (Lentle et al. 2007). This indicated that distension resulted largely from a hydrodynamic effect, i.e. the displacement of fluid in advance of the zone of constriction.

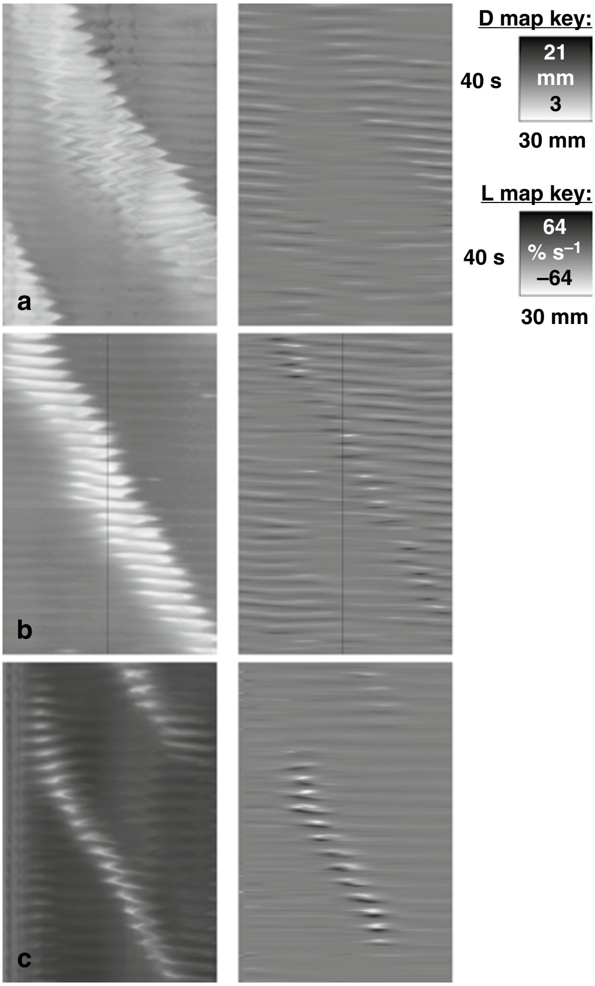
The Zone of Constriction

There is a long-standing controversy regarding the temporal relationship between circular and longitudinal contractions during peristalsis (Lammers et al. 2006). A number of researchers have reported that longitudinal and circular muscles contract synchronously in the small intestine of eutherian species (Bayliss and Starling 1899; Hennig et al. 1999; Smith and Robertson 1998; Stevens et al. 1999; Wood and Perkins 1970; Yokoyama and Ozaki 1990). Further, such concerted contraction occurs in response to 'peristaltic waves, point activation of enteric reflexes and in response to extrinsic nerve stimulation' (Spencer et al. 1999) and 'in a similar manner to concerted contraction occurring during segmentation' (Thuneberg and Peters 2001). Other workers report that longitudinal and circular contractions are out of phase (Grider 2003; Kottegoda 1969; Trendelenburg 1917) during peristalsis suggesting that the two muscle groups are innervated reciprocally (Kottegoda 1969).

The situation is rendered complex by the fact that the progression of radial constriction within peristaltic events takes place in a 'step-like' (Hennig et al. 1999) or pulsatile manner, as do local changes in longitudinal strain (Lentle et al. 2007) (Fig. 8.7). Hence it is necessary to consider whether there is coordination between radial and longitudinal pulses regionally, i.e. considering the entire peristaltic event, or locally, i.e. within the occluded segment. With regard to local coordination, spatiotemporal mapping shows that in the ex vivo possum small intestine pulsatile circular and longitudinal constrictions are in phase, i.e. occur concurrently, within the occluded segment (Fig. 8.8). However, the occluded segment as a whole is lengthened relative to before and after the peristalsis, i.e. has a positive longitudinal strain (Fig. 8.8). The latter lengthening may have been due to the mechanical interaction between muscle layers, which may explain the controversy above.

There is also variation in the duration of individual phasic radial contractile events according to the length of the occluding segment. In those peristaltic events with longer occluding segments, the duration of the pulsatile radial contractions (the width of white stripes in the y dimension) gradually increased while the duration of intervening zones of relaxation (dark stripes) were correspondingly reduced (Fig. 8.7b compared with Fig. 8.7c). In those peristaltic events with the longest

Fig. 8.7 Pairs of spatiotemporal maps showing peristaltic events with different occluded segment lengths in the ex vivo possum ileum: **a** sustained contraction; **b** sustained contraction; and **c** short-lived contraction. The D maps *(left)* show smaller diameters as a lighter shade. On the L map, a darker shade indicates a relaxing (+ve) strain rate and a lighter shade a contracting (–ve) strain rate. (Lentle et al. 2007)



occluding segments, the pulsatile radial contractions became continuous (remained of lighter tone) but modulated in intensity (varied in tone between white and light grey) (Fig. 8.7a). These differences in the duration may result from different levels of excitation in the circular muscle. Thus only in the longest events is the excitation state sufficient to generate sustained contraction of the entire circular musculature in the occluding segment.

Events at the Leading Edge of the Zone of Radial Constriction

The pulsatile longitudinal and radial contractions in spatiotemporal maps of the small intestine have a synchronous jagged appearance at the leading edge or front

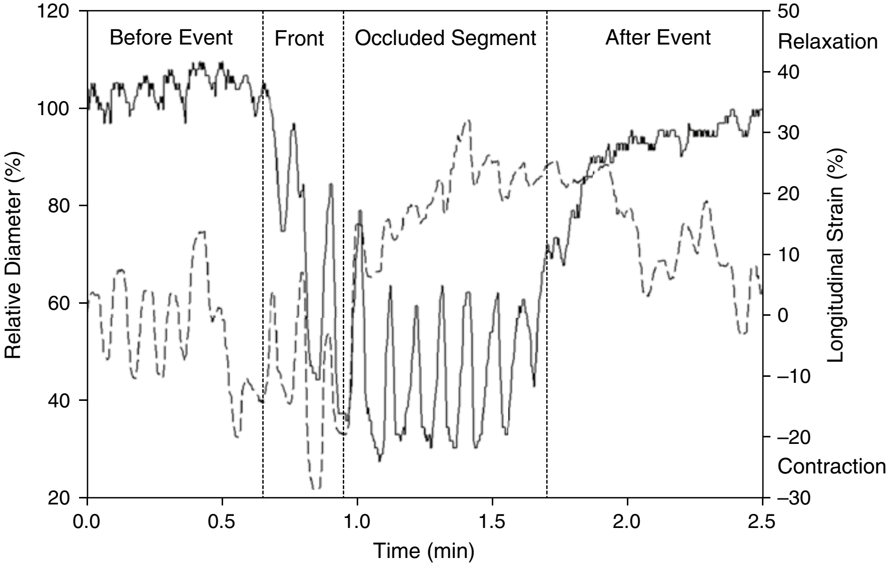
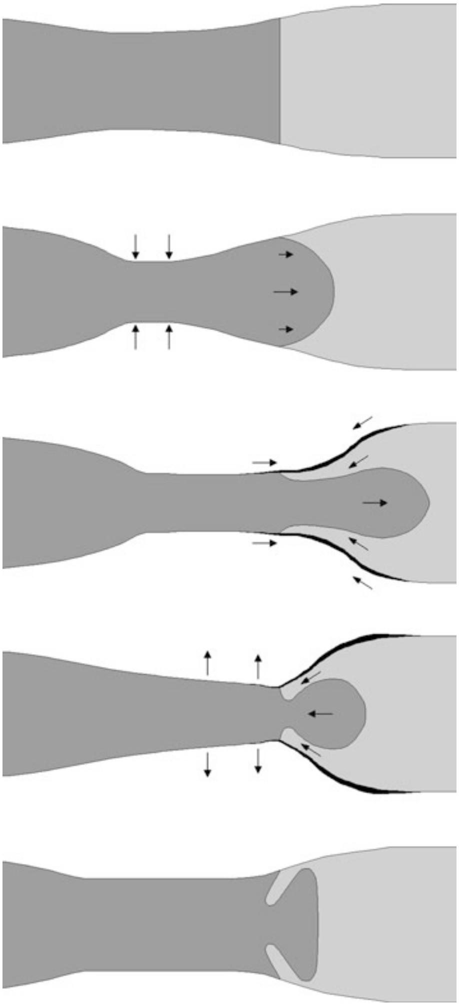


Fig. 8.8 The relationship between diametric *(solid)* and longitudinal *(dashed)* strains during a peristaltic contraction. Measurements taken along the black vertical lines in Fig. 8.7, i.e. they represent the values at a particular location along the preparation. The relative diameter of the intestine was calculated by dividing the actual diameter by the mean diameter and the longitudinal strain by integrating the strain rate. Note that the ileum within the occluded segment is lengthened relative to before and after the peristalsis, but that the higher-frequency component within the occluded segment are in phase in the radial and longitudinal directions. (Lentle et al. 2007)

of the zone of radial constriction (Fig. 8.7) and these phasic longitudinal and radial contractions occur concurrently at this point (Fig. 8.8). As a result, the zone of peristaltic constriction progresses in a series of rhythmic surges rather than as a sustained event of smoothly increasing amplitude. Such a mode of progression fits with current opinion regarding the origin of intestinal muscle contraction, with consecutive slow waves summating to generate successive action potentials (Donnelly et al. 2001) in successive aborad segments (Huizinga 1999). The occurrence of pulsatile rather than smoothly progressing longitudinal constrictions will bring about higher shear rates at the peristaltic front, which will cause the apparent viscosity of pseudoplastic (shear thinning) digesta to be reduced and may facilitate onflow and mixing (Lentle et al. 2005). It is noteworthy that longitudinal contractions are particularly strong at the leading edges of peristaltic events where intraluminal pressure is high, i.e. when the overall tone of the D map was dark (Fig. 8.7). Thus pulsatile longitudinal contractile activity under higher intraluminal pressure may provide a means of alleviating high local pressures generated during pumping of digesta with high apparent viscosity.

It has been proposed that the progression of peristalsis in a series of surges may also promote mixing at the periphery of the lumen (Lentle et al. 2007). Hence coordinated circular and longitudinal waves of muscle contraction may generate mixing

Fig. 8.9 Schematic of suggested mechanism for laminar mixing generated by simultaneous circular and longitudinal contractions during peristalsis. (Lentle et al. 2007)



cells at the front of peristaltic events, which achieve a degree of radial dispersion (Fig. 8.9). Laminar mixing often proceeds by repeated splitting and folding of flow lines (Oldshue 1983). In the proposed mechanism, splitting results from the aboral progression of the peristaltic event causing the location of the mixing cells to be continually displaced while folding results from asymmetry of movements in the intestinal wall.

It is important to note that low-level contractile activity continues during intervals between peristaltic events. Pulsatile longitudinal and radial constrictions continue to occur in spatiotemporal maps taken at times when no peristaltic events were occurring. During these periods, the longitudinal events were of higher amplitude and were out of phase with radial events (Fig. 8.10) indicating there may be mechanical interaction between contracting muscle layers, i.e. passive stretching and

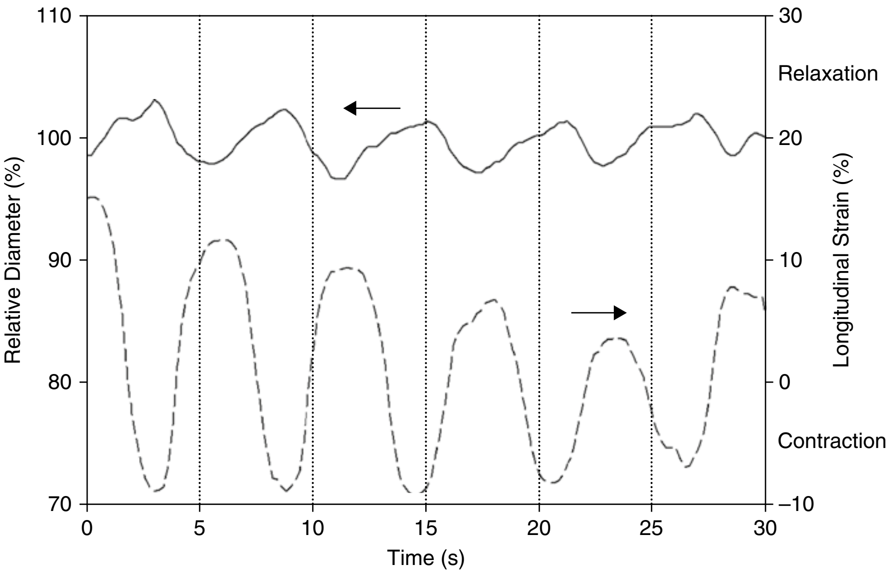


Fig. 8.10 The relationship between diametric *(solid)* and longitudinal *(dashed)* strains during a time when no peristaltic contractions were occurring. Note that the movements in the radial and longitudinal directions are 180° out of phase and the larger amplitude of the longitudinal strains. (Lentle et al. 2007)

stimulation of circular muscle by longitudinal contraction. Such activity may reflect the genesis of segmentative or pendular contractions, or may be an adaptation to reduce apparent viscosity at the margins of the digesta plug.

In certain segments of the intestine, the timing of the peristaltic events and backflow from more distal sites following the termination of these events may intensify mixing. Thus in the duodenum, clusters of peristalses of short duration are provoked by solutions of short-chain fatty acids or by hyperosmolar solutions (Ehrlein et al. 1987). Such ‘clustered contractions’ may continue to arise at the same site, or the site of origin may migrate slowly distad. Similar clustered contractions may also occur in the jejunum and the ileum (Ehrlein et al. 1987). Such activity may be of similar form to segmentative contractions (see below) when coordinated longitudinal and circular phasic contractions rather than isolated contraction of circular muscle (see below) repeatedly arise in adjacent segments but do not propagate causing the contents to be alternately pumped distally and retropulsed.

8.3.2 Segmentation

Segmentative contractions, rhythmic stationary contractions that occur principally in the fed state, were originally reported by Cannon (1912). These events were

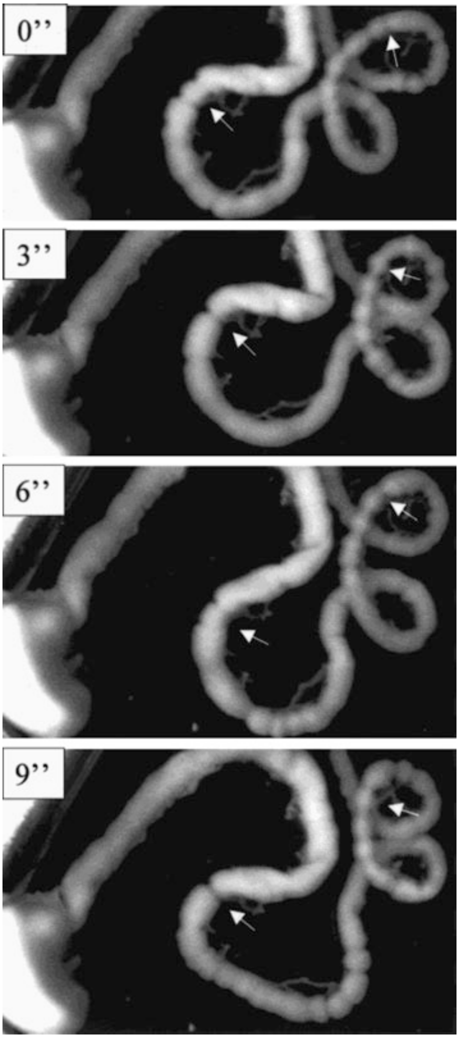


Fig. 8.11 Normal suckling mouse, 5 days pp. Video frames at 3-s intervals illustrating the segmentation frequency. The *arrows* mark the same two points (identifiable by the positions of attached mesenteric blood vessels) in all frames. Note the shifts every 3 s between constriction and dilation. The average distance between constrictions is 1.5 mm. (Thuneberg and Peters 2001)

postulated to result from isolated contractions of the circular muscle layer and to provide a 'default' pattern when propulsive movements, such as peristalsis, were inhibited (Keinke and Ehrlein 1983; Schemann and Ehrlein 1986; Schmid and Ehrlein 1993). There has been debate as to whether the associated muscle contraction is induced by slow wave activity alone (Thuneberg and Peters 2001) or by elements of the ENS, as was originally suggested by Cannon (1912). The simple stationary pattern of alternate, mainly radial, constriction and distension has been shown to occur postprandially in vivo in the adult and suckling mouse (Thuneberg and Peters 2001) (Fig. 8.11). Isolated, i.e. unpaired stationary, circular muscle contractions have been shown to occur in the small intestine of the guinea pig in response to perfusion with decanoic acid (Gwynne et al. 2004). The latter group reported that these segmentative contractions occupied 4–5 mm, lasted 3–7 s, and reduced the outside diameter of the duodenum to a lesser extent than did propulsive contractions. Similar isolated circular muscle contractions, which propagate orad, aborad and bidirectionally, have also been reported (Gwynne et al. 2004). Their speed of propagation (6–8.5 mm/s) was significantly slower than peristaltic contractions that were induced by distension in the same preparation (15–18 mm/s) (Gwynne et al. 2004).

The fact that stationary segmentative contractions comprise mainly circular muscle activity presumably precludes longitudinal contraction from inducing asymmetry at one or other boundaries, as in the case of peristaltic contractions. Published illustrations (Thuneberg and Peters 2001) and spatiotemporal maps (Gwynne et al. 2004) support such an assumption as they show broadly symmetric boundaries, as indeed do short length-propagating segmentative contractions. Hence segmentative contractions may not induce asymmetric vortical flow and effective mixing. However, stationary alternating segmentative contractions will effectively restrict onflow, a function that may be of importance in segments close to the site of gastric outflow and analogous to a physiological sphincter. Thus segmentative activity may contribute to the restriction of gastric onflow, i.e. the ‘duodenal brake’ (Rao et al. 1996).

Further work is needed regarding the effect of the viscosity of the contents on the incidence and characteristics of segmentative contractions. If contraction of a segment of intestine is sufficiently rapid to drive the contents with sufficient force into the relaxed receiving segment so as to generate inertia and vortical flow, and the timing, velocity, and spacing of the subsequent contraction in the receiving segment is sufficient to generate an appropriately sited vortex adjacent to the original vortex, then this may induce local asymmetry of flow and mixing of the contents. The conditions necessary to induce asymmetric vortical flow will depend upon the apparent viscosity of the contents. If the apparent viscosity of the contents is high, then either the velocities of contraction of the adjacent segments must be increased, or the amplitude and timing of the interval between adjacent contractions adjusted, so as to generate sufficient inertia to sequentially establish vortices in the adjacent segments. However, there is little evidence that the velocity, amplitude or timing of segmentative contractions vary in this manner. Indeed, the frequency of ENS pattern generators appears to be relatively fixed being unchanged by the addition of agents that are known to induce changes in responsiveness to neurotransmitters, e.g. histamine (Wood 2006). If the system generating segmentative contractions is inflexible, then this may account for segmentative activity being more evident in the more proximal segments of the small intestine, which contain less viscous digesta (Lentle et al. 2010).

8.3.3 Pendular Activity

Pendular contraction, ‘to-and-fro movements’ or ‘sleeve contractions’ (Thuneberg and Peters 2001) were first described by Bayliss and Starling (1899). They are thought to result from slow-wave-induced (Lammers 2005) isolated contractions in the longitudinal muscle layer (Hennig et al. 1999; Thuneberg and Peters 2001) and commonly occur in the fed state (Thuneberg and Peters 2001). It has been suggested that such contractions may induce local vortical flow (Melville et al. 1975). The general lack of stereotypical pattern (Christensen 1993) and high degree of asymmetry of pendular contractions seem well suited to the generation of vortical mixing. The longitudinal contractile activity does not appear to be distributed evenly around the radius of the gut often inducing local indentations of the abmesenteric intestinal wall (Thuneberg and Peters 2001). Variations in the direction and propagation of longitudinal contractions are coupled with those of the slow waves, which similarly vary in origin and direction (Lammers 2005; Stevens et al. 2000). Further, they are associated with ‘conspicuous wrinkling of the serosal surface’ (Thuneberg and Peters 2001), which may constitute the formation of plicae circulares (DeSesso and Jacobson 2001) and further facilitate the development of local turbulence at the wall. Such wrinkling may, as in the case of contractions of the circular muscle layer, reflect the vectoring of residual stress into the mucosal layer, and it seems more likely to contribute to the formation of plicae given that longitudinally cut strips of living small intestinal wall bend outwards (Dou et al., unpublished data in Gregersen 2003).

Recent work indicates that low-amplitude cyclic longitudinal contractions also occur during physiological ileus, i.e. the intervals between peristaltic contractions, with a frequency of 9.2 cycles/min in brushtail possums (Lentle et al. 2007). It has been suggested that this contractile pattern may induce local shear thinning of digesta, and hence facilitate plug flow (Lentle et al. 2007). Were these contractions asymmetric in their action they could also engender mixing.

References

Allen ML, Zamani S, Dimarino AJ (2003) The effect of gravity on oesophageal peristalsis inhumans. Neurogastroenterol Mot 9:71-76

Alvarez WC, Zimmermann A (1927) The absence of inhibition ahead of peristaltic rushes. Am J Physiol 83:52-59

Anderson DJ, Hector MP, Linden RW (1985) The possible relation between mastication and parotid secretion in the rabbit. J Physiol 364:19-29

Balaban DH, Yamamoto Y, Liu J, Pehlivanov N, Wisniewski R, DeSilvey D, Mittal RK (1999) Sustained esophageal contraction: A marker of esophageal chest pain identified by intraluminal ultrasonography. Gastroenterology 116:29-37

Bayliss WM, Starling EH (1899) The movements and innervation of the small intestine. J Physiol 24:99-143

Beyak MJ, Collman PI, Valdez DT, Xue S, Diamant NE (1997) Superior laryngeal nerve stimulation in the cat: Effect on oropharyngeal swallowing, oesophageal motility and lower oesophageal sphincter activity. Neurogastroenterol Mot 9:117-127

Boeckxstaens GE (2005) The lower oesophageal sphincter. Neurogastroenterol Mot 17:13-21

Bogeski G, Shafton AD, Kitchener PD, Ferens DM, Furness JB (2005) A quantitative approach to recording peristaltic activity from segments of rat small intestine in vivo. Neurogastroenterol Mot 17:262-272

Bornstein JC, Furness JB, Kunze WAA, Bertrand PP (2002) Enteric reflexes that influence motility. In: Brookes SJH, Costa M (eds) Innervation of the gastrointestinal tract. Taylor & Francis, London

Bosma JF (1957) Deglutition: Pharyngeal stage. Physiol Rev 37:275-300

Brookes S, Costa M (2002) Innervation of the gastrointestinal tract. Taylor & Francis, London

Burns JC, Parkes T (1967) Peristaltic motion. J Fluid Mech 29:731-743

Buthpitiya AG, Stroud D, Russell COH (1987) Pharyngeal pump and esophageal transit. Dig Dis Sci 32:1244-1248

Cannon WB (1902) The movements of the intestines studied by means of the roentgen rays. Am J Physiol 6:251-277

Cannon WB (1912) Peristalsis, segmentation, and the myenteric reflex. Am J Physiol 30:114-128

Cannon WB (1906) The motor activities of the stomach and small intestine after splanchnic and vagus section. Am J Physiol 17:429-442

Castelain C, Mokrani A, Legentilhomme P, Peerhossaini H (1997) Residence time distribution in twisted pipe flows: helically coiled system and chaotic system. Exp Fluids 22:359–368

Christensen J (1993) The enteric nervous system. In: Kumar D, Wingate D (eds) An illustrated guide to gastrointestinal motility. Churchill Livingstone, Edinburgh, pp 10-31

Clouse R, Diamant N (2006) Motor function of the esophagus. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Academic Press, San Diego pp 913–926

Cook IJ, Dodds WJ, Dantas RO, Massey B, Kern MK, Lang IM, Brasseur JG, Hogan WJ (1989) Opening mechanisms of the human upper esophageal sphincter. Am J Physiol 257:G748-759

Costa M, Brookes SJH, Hennig GW (2000) Anatomy and physiology of the enteric nervous system. Gut 47:iv15-iv19

Dantas RO, Dodds WJ, Massey BT, Shaker R, Cook IJ (1990) Manometric characteristics of glossopalatal sphincter. Dig Dis Sci 35:161-166

De Backer O, Blanckaert B, Leybaert L, Lefebvre RA (2008) A novel method for the evaluation of intestinal transit and contractility in mice using fluorescence imaging and spatiotemporal motility mapping. Neurogastroenterol Mot 20:700-707

DeSesso JM, Jacobson CF (2001) Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats. Food Chem Toxicol 39:209-228

Dixon AD (1963) Nerve plexuses in the oral mucosa. Arch Oral Biol 8:435-444

Dodds WJ, Christensen J, Dent J, Wood JD, Arndorfer RC (1978a) Esophageal contractions induced by vagal stimulation in the opossum. Am J Physiol 235:E392-401

Dodds WJ, Hogan WJ, Reid DP, Stewart ET, Arndorfer RC (1973) A comparison between primary esophageal peristalsis following wet and dry swallows. J Appl Physiol 35:851-857

Dodds WJ, Stef JJ, Stewart ET, Hogan WJ, Arndorfer RC, Cohen EB (1978b) Responses of feline esophagus to cervical vagal stimulation. Am J Physiol 235:E63-73

Dodds WJ, Stewart ET, Logemann JA (1990) Physiology and radiology of the normal oral and pharyngeal phases of swallowing. Am J Roentgenol 154:953-963

Donnelly G, Jackson TD, Ambrous K, Ye J, Safdar A, Farraway L, Huizinga JD (2001) The myogenic component in distention-induced peristalsis in the guinea pig small intestine. Am J Physiol 280:G491-500

Ehrlein HJ, Schemann M, Siegle ML (1987) Motor patterns of small intestine determined by closely spaced extraluminal transducers and videofluoroscopy. Am J Physiol 253:G259-267

Ferguson DB, Shuttleworth A, Whittaker DK (1999) Oral bioscience. Churchill Livingstone, Edinburgh

Frieling T, Hermann S, Kuhlbusch R, Enck P, Silny J, Lübke HJ, Strohmeyer G, Haeussinger D (1996) Comparison between intraluminal multiple electric impedance measurement and manometry in the human oesophagus. Neurogastroenterol Mot 8:45-50

Frøkjær JB, Andersen SD, Drewes AM, Gregersen H (2006) Ultrasound-determined geometric and biomechanical properties of the human duodenum. Dig Dis Sci 51:1662-1669

Gärtner K (2001) The forestomach of rats and mice, an effective device supporting digestive metabolism in muridae (review). J Exp Anim Sci 42:1-20

Gregersen H (2003) Biomechanics of the gastrointestinal tract: New perspectives in motility research and diagnostics. Springer Verlag, New York

Gregersen H, Lee TC, Chien S, Skalak R, Fung YC (1999) Strain distribution in the layered wall of the esophagus. J Biomech Eng 121:442-448

Grider JR (2003) Reciprocal activity of longitudinal and circular muscle during intestinal peristaltic reflex. Am J Physiol 284:G768-775

Gwynne RM, Thomas EA, Goh SM, Sjovall H, Bornstein JC (2004) Segmentation induced by intraluminal fatty acid in isolated guinea-pig duodenum and jejunum. J Physiol 556:557-569

Hasegawa A, Otoguro A, Kumagai H, Nakazawa F (2005) Velocity of swallowed gel food in the pharynx by ultrasonic method. Nippon Shokuhin Kagaku Kogaku Kaishi 52:441-447

Hennig GW, Costa M, Chen BN, Brookes SJH (1999) Quantitative analysis of peristalsis in the guinea-pig small intestine using spatio-temporal maps. J Physiol 517:575-590

Hiiemae K, Heath MR, Heath G, Kazazoglu E, Murray J, Sapper D, Hamblett K (1996) Natural bites, food consistency and feeding behaviour in man. Arch Oral Biol 41:175-189

Hollis JB, Castell DO (1975) Effect of dry swallows and wet swallows of different volumes on esophageal peristalsis. J Appl Physiol 38:1161-1164

Huizinga JD (1999) Gastrointestinal peristalsis: Joint action of enteric nerves, smooth muscle, and interstitial cells of Cajal. Microsc Res Tech 47:239-247

Huizinga JD, Lammers WJEP (2009) Gut peristalsis is governed by a multitude of cooperating mechanisms. Am J Physiol 296:G1-8

Hutchings JB, Lillford PJ (1988) The perception of food texture - the philosophy of the breakdown path. J Text Stud 19:103-115

Jean A (2001) Brain stem control of swallowing: Neuronal network and cellular mechanisms. Physiol Rev 81:929-969

Jean A, Dallaporta M (2006) Electrophysiologic characterization of the swallowing pattern generator in the brainstem. GI Motility Online, doi:101038/gimo9

Jeffrey B, Udaykumar HS, Schulze KS (2003) Flow fields generated by peristaltic reflex in isolated guinea pig ileum: Impact of contraction depth and shoulders. Am J Physiol 285:G907-918

Kamm MA, Van Der Sijp JR, Lennard-Jones JE (1992) Colorectal and anal motility during defaecation. Lancet 339:820

Keinke O, Ehrlein HJ (1983) Effect of oleic acid on canine gastroduodenal motility, pyloric diameter and gastric emptying. Q J Exp Physiol 68:675-686

Klineberg I (1980) Influences of temporomandibular articular mechanoreceptors on functional jaw movements. J Oral Rehabil 7:307-317

Knutson T, Fridblom P, Ahlstro m H, Magnusson A, Tannergren C, Lennernas H (2009) Increased understanding of intestinal drug permeability determined by the LOC-I-GUT approach using multislice computed tomography. Mol Pharmaceut 6:2-10

Kottegoda SR (1969) An analysis of possible nervous mechanisms involved in the peristaltic reflex. J Physiol 200:687-712

Lammers WJEP (2005) Spatial and temporal coupling between slow waves and pendular contractions. Am J Physiol 289:G898-903

Lammers WJEP, Lammers-van den Berg AM, Morrison JFB, Petroianu GA (2006) Translating trendelenburg; back to the future. Naunyn Schmiedebergs Arch Pharmacol 373:134-138

Latham TW (1966) Fluid motions in a peristaltic pump. MS Thesis, Massachusetts Institute of Technology, Cambridge, MA

Lentle RG, Hemar Y, Hall CE (2006) Viscoelastic behaviour aids extrusion from and reabsorption of the liquid phase into the digesta plug: Creep rheometry of hindgut digesta in the common brushtail possum trichosurus vulpecula. J Comp Physiol B176:469-475

Lentle RG, Hemar Y, Hall CE, Stafford KJ (2005) Periodic fluid extrusion and models of digesta mixing in the intestine of a herbivore, the common brushtail possum (trichosurus vulpecula). J Comp Physiol B175:337-347

Lentle RG, Janssen PWM, Asvarujanon P, Chambers P, Stafford KJ, Hemar Y (2007) High definition mapping of circular and longitudinal motility in the terminal ileum of the brushtail possum trichosurus vulpecula with watery and viscous perfusates. J Comp Physiol B177:543-556

Lentle RG, Janssen PWM, Goh K, Chambers P, Hulls C (2010) Quantification of the effects of the volume and viscosity of gastric contents on antral and fundic activity in the rat stomach maintained ex vivo. Dig Dis Sci DOI 10.1007/s10620-010-1164-y:1-12

Li D, Guo M (2007) Morphology of the levator ani muscle. Dis Colon Rectum 50:1831-1839

Lillford PJ (1991) Texture and acceptability of human foods. In: Vincent JFV, Lillford PJ (eds) Feeding and the texture of foods. Cambridge University Press, Cambridge, pp 231–243

Linden P, Tippett D, Johnston J, Siebens A, French J (1989) Bolus position at swallow onset in normal adults: Preliminary observations. Dysphagia 4:146-150

Linden RWA (1990) Periodontal mechanoreceptors and their functions. In: Taylor A (ed) Neurophysiology of the jaws and teeth. Macmillan, New York, pp 52-95

Lu X, Gregersen H (2001) Regional distribution of axial strain and circumferential residual strain in the layered rabbit oesophagus. J Biomech 34:225-233

Lucas P (2004) Dental functional morphology. Cambridge University Press, Cambridge

Lund J (1991) Mastication and its control by the brain stem. Crit Rev Oral Biol Med 2:33

Lund JP, Kolta A (2006) Generation of the central masticatory pattern and its modification by sensory feedback. Dysphagia 21:167-174

Macagno EO, Christensen JM (1981) Fluid mechanics of gastrointestinal flow. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Raven, New York, pp 335-358

Marciani L, Gowland PA, Spiller RC, Manoj P, Moore RJ, Young P, Fillery-Travis AJ (2001) Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. Am J Physiol 280:G1227-1233

McRorie J, Brown S, Cooper R, Givaruangsawat S, Scruggs D, Boring G (2000) Effects of dietary fibre and olestra on regional apparent viscosity and water content of digesta residue in porcine large intestine. Aliment Pharmacol Ther 14:471–477

Melville J, Macagno E, Christensen J (1975) Longitudinal contractions in the duodenum: Their fluid-mechanical function. Am J Physiol 228:1887-1892

Meng Y, Rao MA, Datta AK (2005) Computer simulation of the pharyngeal bolus transport of Newtonian and non-Newtonian fluids. Food Bioproducts Process 83:297-305

Miller JL, Watkin KL (1996) The influence of bolus volume and viscosity on anterior lingual force during the oral stage of swallowing. Dysphagia 11:117-124

Misra JC, Pandey SK (2001) A mathematical model for oesophageal swallowing of a food-bolus. Math Comput Model 33:997-1009

Mittal RK, Liu J, Puckett JL, Bhalla V, Bhargava V, Tipnis N, Kassab G (2005) Sensory and motor function of the esophagus: Lessons from ultrasound imaging. Gastroenterology 128:487-497

Mittal RK, Padda B, Bhalla V, Bhargava V, Liu J (2006) Synchrony between circular and longitudinal muscle contractions during peristalsis in normal subjects. Am J Physiol 290:G431-438

Mokrani A, Castelain C, Peerhossaini H (1997) Effects of chaotic advection on heat transfer. Int J Heat Mass Transf 40:3089–3104

Nguyen HN, Silny J, Albers D, Roeb E, Gartung C, Rau G, Matern S (1997) Dynamics of esophageal bolus transport in healthy subjects studied using multiple intraluminal impedancometry. Am J Physiol 273:G958-964

Nicosia MA, Brasseur JG, Liu JB, Miller LS (2001) Local longitudinal muscle shortening of the human esophagus from high-frequency ultrasonography. Am J Physiol 281:G1022-1033

Nicosia MA, Robbins JA (2001) The fluid mechanics of bolus ejection from the oral cavity. J Biomech 34:1537-1544

Okada A, Honma M, Nomura S, Yamada Y (2007) Oral behavior from food intake until terminal swallow. Physiol Behav 90:172-179

Oldshue JY (1983) Fluid mixing technology. Chemical Engineering, New York

Palmer JB (1998) Bolus aggregation in the oropharynx does not depend on gravity. Arch Phys Med Rehabil 79:691-696

Palmer JB, Rudin NJ, Lara G, Crompton AW (1992) Coordination of mastication and swallowing. Dysphagia 7:187-200

Paphangkorakit J, Osborn JW (2000) The effect of normal occlusal forces on fluid movement through human dentine in vitro. Arch Oral Biol 45:1033-1041

Patel PD, Picologlou BF, Lykoudis PS (1973) Biorheological aspects of colonic activity. II. Experimental investigation of the rheological behavior of human feces. Biorheology 10:441-445

Paterson WG (1999) Alteration of swallowing and oesophageal peristalsis by different initiators of deglutition. Neurogastroenterol Mot 11:63-67

Prinz JF, Lucas PW (1997) An optimization model for mastication and swallowing in mammals. Proc R Soc Lond B Biol Sci 264:1715-1721

Rao SS, Lu C, Schulze-Delrieu K (1996) Duodenum as a immediate brake to gastric outflow: A videofluoroscopic and manometric assessment. Gastroenterology 110:740-747

Robbins J, Hamilton JW, Lof GL, Kempster GB (1992) Oropharyngeal swallowing in normal adults of different ages. Gastroenterology 103:823-829

Roman C, Gonella J (1987) Extrinsic control of digestive tract motility. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Raven, New York, pp 507-553

Saitoh E, Shibata S, Matsuo K, Baba M, Fujii W, Palmer JB (2007) Chewing and food consistency: Effects on bolus transport and swallow initiation. Dysphagia 22:100-107

Schemann M, Ehrlein HJ (1986) Postprandial patterns of canine jejunal motility and transit of luminal content. Gastroenterology 90:991-1000

Schmid HR, Ehrlein HJ (1993) Effects of enteral infusion of hypertonic saline and nutrients on canine jejunal motor patterns. Dig Dis Sci 38:1062-1072

Schulze-Delrieu K (1999) Visual parameters define the phase and the load of contractions in isolated guinea pig ileum. Am J Physiol 276:G1417-1424

Schulze KS, Clark E (2008) Ink dispersion by sequential contractions in isolated segments of guinea pig ileum and duodenum. Neurogastroenterol Mot 20:1317-1327

Seo HS, Hwang IK, Han TR, Kim IS (2007) Sensory and instrumental analysis for slipperiness and compliance of food during swallowing. J Food Sci 72:S707-S713

Shafik A (1980) A new concept of the anatomy of the anal sphincter mechanism and the physiology of defecation: IX. Single loop continence: A new theory of the mechanism of anal continence. Dis Colon Rectum 23:37-46

Shafik A (1991) Levator-puborectalis reflex: Its role in elimination. Pract Gastroenterol 15:28-35

Shafik A (1993) Anorectal tightening reflex: Role in fecal incontinence. Eur Surg Res 25:399-405

Shafik A, Shafik AA, El-Sibai O, Ali YA (2003) Videodefecography: A study of the rectal motile pattern. Surg Radiol Anat 25:139-144

Shaker R, Ren J, Podvrsan B, Dodds WJ, Hogan WJ, Kern M, Hoffmann R, Hintz J (1993) Effect of aging and bolus variables on pharyngeal and upper esophageal sphincter motor function. Am J Physiol 264:G427-432

Smith TK, Robertson WJ (1998) Synchronous movements of the longitudinal and circular muscle during peristalsis in the isolated guinea-pig distal colon. J Physiol 506:563-577

Spencer NJ, Walsh M, Smith TK (1999) Does the guinea-pig ileum obey the ‘law of the intestine’? J Physiol 517:889-898

Srinivasan R, Vela MF, Katz PO, Tutuian R, Castell JA, Castell DO (2001) Esophageal function testing using multichannel intraluminal impedance. Am J Physiol 280:G457-462

Steele CM, Cichero JAY (2008) A question of rheological control. Dysphagia 23:199-201

Steffe JF (1996) Rheological methods in food process engineering. Freeman Press, East Lancing

Stevens RJ, Publicover NG, Smith TK (1999) Induction and organization of Ca2+ waves by enteric neural reflexes. Nature 399:62-66

Stevens RJ, Publicover NG, Smith TK (2000) Propagation and neural regulation of calcium waves in longitudinal and circular muscle layers of guinea pig small intestine. Gastroenterology 118:892-904

Sugarbaker DJ, Rattan S, Goyal RK (1984a) Mechanical and electrical activity of esophageal smooth muscle during peristalsis. Am J Physiol 246:G145-150

Sugarbaker DJ, Rattan S, Goyal RK (1984b) Swallowing induces sequential activation of esophageal longitudinal smooth muscle. Am J Physiol 247:G515-519

Takahashi T, Nitou T, Tayama N, Kawano AKI, Ogoshi H (2002) Effects of physical properties and oral perception on transit speed and passing time of semiliquid foods from the mid-pharynx to the hypopharynx. J Text Stud 33:585-598

Thuneberg L, Peters S (2001) Toward a concept of stretch-coupling in smooth muscle. I. Anatomy of intestinal segmentation and sleeve contractions. Anat Rec 262:110-124

Trendelenburg P (1917) Physiologische und pharmakologische versuche über die dünndarmperistaltik. Naunyn Schmiedebergs Arch Pharmacol 81:55-129

Vincent JFV (1990) Fracture properties of plants. Adv Bot Res 17:235–287

Vincent JFV, Jeronimidis G, Khan AA, Luyten H (1991) The wedge fracture test a new method for measurement of food texture. J Text Stud 22:45-57

Waterman SA, Costa M, Tonini M (1994) Accommodation mediated by enteric inhibitory reflexes in the isolated guinea-pig small intestine. J Physiol 474:539-546

Weinberg SL, Eckstein EC, Shapiro AH (1971) An experimental study of peristaltic pumping. J Fluid Mech 49:461-479

Wise JL, Murray JA, Conklin JL (2004) Regional differences in oesophageal motor function. Neurogastroenterol Mot 16:31-37

Wood JD (2006) Integrative functions of the enteric nervous system. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Academic Press, San Diego, pp 665–684

Wood JD, Perkins WE (1970) Mechanical interaction between longitudinal and circular axes of the small intestine. Am J Physiol 218:762-768

Yokoyama S, Ozaki T (1990) Contractions of the longitudinal and circular muscle of the small intestine. Prog Clin Biol Res 327:483-492

Zbar AP, Guo M, Pescatori M (2008) Anorectal morphology and function: Analysis of the Shafik legacy. Techniques in Coloproctology 12:191-200

Chapter 9
Local Motility and Flow in Segments that Exhibit Volume Retention

Contents

9.1 Introduction

The morphological differentiation of the fore-, mid- and hindgut is established early in the embryonic development of a range of species (Kiefer 2003; Stevens and Hume 1995). However, subsequent differentiation requires that the mechanical function of the three divisions be efficiently integrated, notably in regard to their volumetric capacity. Hence, the functions of mixing and propulsion are complicated in the stomach and colon of vertebrates by a requirement for volume retention. Indeed, the distinctive gross morphology of these segments may arise largely from this latter requirement. Given that the foregut of macropods is remarkably similar in morphology to the sacculated colon of humans (Hume 1999) and differs from the uniloculate form of the human stomach, it seems that either sacciform or fusiform morphology can be equally successful in providing gastric volume retention.

Volume retention may be required for a number of reasons. The strategies of intermittent feeding and defecation may be useful in predator avoidance, but require volume retention of food or faeces to span the interval between these activities. The most proximal and distal segments of the gastrointestinal tract are best situated to act as reservoirs to enable the development of these behavioural strategies. Volume

R. G. Lentle, P. W. M. Janssen, *The Physical Processes of Digestion*,
DOI 10.1007/978-1-4419-9449-3_9, © Springer Science+Business Media, LLC 2011

retention may also be required in relation to the speed at which solution, digestion and/or absorption of nutrients can be conducted. Microbial digestion is a tardy process relative to enzymatic digestion (Chivers and Langer 1994). Given the role of the gut wall in limiting the penetration of pathogens and its consequent complex morphology, there may be occasions when these adaptations limit the rates at which particular nutrients may be absorbed. This may necessitate a prolongation of residence time or reduction in rate of onflow of digesta to allow efficient absorption of nutrients in the containing or subsequent segment.

The efficient operation of the stomach and colon in the face of an additional requirement for volume retention requires complex solutions to problems of flow and mixing, which have necessitated extensive morphological and physiological divergence from the form and function of a simple distended tube. As noted earlier, the increased distance between the opposing walls of a distended sacculate structure requires greater amplitude of phasic contraction in order to secure occlusion than in a simple tubular structure, such as the small intestine. Indeed, this may have led to a general tapering of the distal ends of sacculate digestive structures so that greater occlusion from a distally travelling phasic contraction is more likely in this region, allowing larger propulsive and triturative forces to be exerted. In the blind-ending caeca of the rabbit (Ehrlein 1979) and those of birds (Janssen et al. 2009), the increasing pressure generated at the tip of the taper by the distally progressing phasic contraction may lead to the generation of a subsequent proximally progressing contraction, which mixes the lumen contents prior to institution of another triturative cycle.

9.2 The Colon

The apparent viscosity of digesta from the distal colon is generally greater than that from the small intestine (Lentle et al. 2005). According to the Hagen–Poiseuille law, greater viscosity results in greater forces being required to maintain the same volumetric flow rate through a simple conduit. The same equation indicates that relatively small increases in the radius of the conduit will dramatically reduce the force required, as flow rate is proportional to the fourth power of the radius. Hence increasing the radius of a given length of colon will lower the force required to propel the more viscous contents as well as increasing its overall volume.

The functional capacity of the colon can only be increased by an increase in its radius if there is concomitant slowing in the net rate of propulsion with respect to that in the small intestine. Such slowing of the transit of digesta would allow greater time for the fermentative digestion of any material that is not amenable to enzymatic digestion in the proximal bowel. A reduction in colonic transit time could be achieved either by reducing the frequency or the rate of propagation of colonic phasic contractions with respect to those in the small intestine, or by altering the direction of propagation. Hence the maximal frequency of colonic peristaltic contractions is around half that in the ileum in the pig (Hipper and Ehrlein 2001), while net colonic transit rate may be reduced in hindgut fermenting species, such as rabbits

(Ehrlein and Ruoff 1982; Fioramonti and Ruckebusch 1974a, b, 1976) and horses (Ross et al. 1990), by intermittent proximally propagating contractions. It is worthy of note in regard to the latter strategy that proximally propagating peristalsis may also occur in the small intestine (Hennig et al. 1999; Lammers et al. 2002), although the predominant direction of propagation is distad (Lammers et al. 2002). Therefore, the proximad contractions must occur more frequently in the colon if they are to be successful in reducing transit time with respect to that of the small intestine.

While an increase in colonic diameter may facilitate pumping of viscid digesta and increase transit time, it may reduce the efficiency of mixing. The mass transfer path of nutrients to the mucosa will be longer in a larger diameter tube and plug flow due to slip at the wall is more likely. A reduction in the frequency of peristaltic contractions in order to prolong colonic transit time will reduce the speed of mixing, the rate of admixture of digesta with resident enteral microbiota, and the consequent rates of liberation and absorption of the products of microbial fermentation. A strategy of exerting great contractile force to induce turbulent mixing in viscous digesta is probably not tenable given the lower potential yield of nutrients from hindgut digesta. In summary, an increase in the capacity of the colon and the apparent viscosity of its contents, along with the slowing in transit time that is necessary for efficient fermentative digestion, require the establishment of mixing and digestive strategies that differ from those to those found in the proximal intestine.

The significant differences in the disposition and organisation of interstitial cells of Cajal (ICCs) in the wall of the colon from those in the wall of the small intestine (Pluja et al. 2001; Smith et al. 1987), and the range of contractile activities exhibited by the colon (Ehrlein et al. 1982, 1983; Lentle et al. 2008; Schulze-Delrieu et al. 1996a) are testament to the degree of functional specialisation that has developed to overcome these difficulties. In a number of species, functional specialisation has occurred in conjunction with that in the caecum so that whole digesta or nutrient-rich fractions of digesta can be displaced from the proximal colon into a blind-ended extension of the caecum where they are temporarily sequestered allowing additional time for fermentative digestion and the absorption of nutrient products (Stevens and Hume 1995).

Additional morphological specialisation is evident in a number of species, including man, where the bulk of the longitudinal muscle is condensed into taeniae, flat bands of muscle that run lengthwise along the proximal colon. These structures have evolved independently at a number of different sites in different species, e.g. the stomachs of macropods and colobine monkeys (Langer 1988), yet the purpose of such adaptation is unclear (Jacobshagen 1937; Langer 1988; Weissberg 1937). One hypothesis relates to the need for efficient volume retention. Studies of changes in the cross-sectional area of the colonic lumen with the transition from a tubular to various sacculated geometries containing increasing numbers of taeniae indicate that the cross-sectional area, and hence the volume of contained digesta per unit wall length, is greatest in tubes that bear three taeniae and a corresponding number of out-pouchings between them (Langer 1988). Other hypotheses are based on the idea that taeniae act as firm longitudinal cables for the attachment of circular muscles (Meyers et al. 1973) so that concerted contractions of the latter can reduce colonic cross-sectional area without generating unduly high intraluminal pressure (Langer 1988). Hence, successive bands of circular muscle contraction form the boundaries of haustral folds which advance slowly towards the rectum generating plug flow of viscous or semisolid digesta. As each circular constriction migrates distally, the locally infolded colonic wall induces displacement of digesta. The accommodation of the distally displaced digesta is facilitated by the greater compliance of haustral walls that contain no longitudinal muscle.

In some species, segments of the colon may operate to selectively sequester the liquid phase or to selectively retain finer particles, particularly in small-bodied animals whose overall mean retention time (MRT) of digesta is necessarily short (Cork et al. 1999), and in animals that have specialised in the processing of fibrous foods (Björnhag 1994; Karasov and Hume 1997). The operation of such colonic particle sorting mechanisms (CSM) has been deduced principally from the relative rates of movement of the liquid and the coarse particulate fractions of digesta (Björnhag 1972). The term 'colonic sorting mechanism' is somewhat misleading as the process must both sort particles and selectively retain the finer fraction, be they nutrient-rich particles (retained for their more complete digestion) or fermentative bacteria (retained to increase digestive efficiency). The underlying mechanisms have not yet been fully described. Terms such as 'pressing out' (Sakaguchi 2003) or 'squeezing' (Hörnicke and Björnhag 1980) have been used in descriptions of CSMs (Björnhag 1981; Sperber et al. 1983), but such process would only serve to express the liquid phase along with some finer particles. There are no clear descriptions of the manner in which the fluid, and particulate fractions within it, are selectively retained. Thus it is not known whether there is simply a selective retention of the liquid phase and any contained fine particles, or whether specific patterns of colonic motility, such as the 'rolling' movements within colonic haustra, in some way entrain the fine particles within the fluid phase (Ehrlein et al. 1982) and prevent them from being reabsorbed into the digesta plug during the process of creep recovery on cessation of contraction (Lentle et al. 2005). It is noteworthy that several species with a haustrated colon have been reported to selectively retain fine particles but that humans have not.

The contractile activity of the healthy human colon appears to vary diurnally when monitored by manometric means. The bulk of propagating contractile sequences occur during the day, often after the consumption of a meal, and move aborad. However, the colonic motility in humans with habitual constipation is distributed more evenly through the 24-h period, and comprises greater proportions of orally propagating and less sustained sequences (Dinning et al. 2008). In the latter case, it appears that the transit time is reduced, the water absorption is more extensive, and faecal consistency is correspondingly harder.

9.2.1 Colonic Movements

The complexity of human colonic movement has been documented in a number of radiographic (Ritchie 1968a, b; Ritchie et al. 1971), manometric (Lemann et al.

1995), and electrophysiological (Sarna et al. 1982) studies but there is limited information regarding the effect of tone or phasic movements on the flow of colonic digesta, either in human or animal studies. Further, the description of the various types of movement has been limited until recently by methodology. Early studies of variation in lumen pressure were limited by the fact that fluctuations were recorded only during strong (significantly occlusive) contractions and not during non-occlusive movements (Sarna et al. 1982; Sasaki et al. 1996). Again, the limited view of the lumen obtained by radiographic methods and the poor resolution restricted accurate quantitative measurement of movements of the colonic wall (Cannon 1902). The advent of spatiotemporal mapping (D'Antona et al. 2001; Hennig et al. 1999) has allowed more detailed study of the volume changes that accompany such movements, notably those in the proximal colon of the rabbit, which is triply haustrated and of similar morphology to that of humans (Lentle et al. 2008).

Three broad patterns of phasic contractile activity were originally described in the proximal colon of the rabbit: slowly aborad migrating rings of deep circular constriction; ongoing high-frequency contractions of the circular musculature within the haustra; and sporadic monophasic, sustained, large-amplitude contractions of the longitudinal and circular musculature that rapidly propagated down the length of the colon (Ehrlein et al. 1982). A fourth type of contraction, an ongoing, rapidly propagating, shallow event was described recently and termed the fast phasic contraction (Lentle et al. 2008). Three types of movement similar to those exhibited by the rabbit colon have been identified in humans: mass peristalsis (Holzknecht 1909); movements associated with haustra (Ritchie 1968a; Schwarz 1911); and small rapid movements (Hertz and Newton 1913). Two of these four types of contraction, i.e. haustral progression and mass peristalsis, appear to be neurally mediated (Lentle et al. 2008), while colonic rippling and fast phasic activity are resistant to nerve-blocking agents, and hence likely to be mediated by smooth muscle cells and ICCs (Cannon 1912; Mackenna and McKirdy 1972). In the rabbit, orad and aborad mass peristalses of the caecum are coordinated with caecal filling from the small intestine and with caecal emptying into the colon (Ehrlein 1979). Further, these activities are coordinated with orad and aborad haustral progression in the colon and with aborad pumping activity in the terminal ileum and sacculus rotundus (Ehrlein 1979).

Colonic tone is reported to increase in human subjects after a meal, as evidenced by the long-term reduction in the volume of intracolonic balloon catheters (Coulie et al. 2001; Ford et al. 1995). The tonal increase depends on the fasting volume and caloric intake, and the increase was greater in the proximal than in the sigmoid colon (Ford et al. 1995). It has been postulated that this tonal increase serves to narrow the lumen, and facilitate mixing and propulsion (Sarna and Shi 2006). However, this presupposes the contents are sufficiently fluid to undergo mixing. In regions where the contents are viscous or semisolid, such reduction will render propulsion more difficult for reasons discussed earlier. Hence the greater increase in the tone of the proximal colon than in the sigmoid colon may reflect adaptation to differences in the physical properties of the contents.

9.2.1.1 Mass Peristalsis

A pattern of colonic contraction variously termed 'mass peristalsis' (Ehrlein et al. 1982; Lentle et al. 2008) and 'giant migrating contraction' (Sarna and Shi 2006) has been identified in a number of species including humans (Sarna and Shi 2006). Mass peristaltic events occur irregularly and can generate high pressure in the colonic lumen (Ehrlein et al. 1982, 1983), e.g. intraluminal pressure rising to over 223 mm Hg in humans (Rao et al. 2001). The frequency of mass peristalses increases postprandially (Coulie et al. 2001; Ford et al. 1995). Mass peristalsis resembles small intestinal peristalsis in that there is coordinated contraction of circular and longitudinal muscle albeit in morphologically separate sites. Mass peristaltic events are the only colonic contractile events to be preceded by a zone of descending neural inhibition and relaxation of tone to produce local dilatation (Bassotti et al. 1999; Chey et al. 2001; Ehrlein et al. 1982, 1983; Karaus and Sarna 1987; Lentle et al. 2008). Distally progressing mass peristaltic events inhibit the action of the internal anal sphincter thus promoting defecation (Bassotti et al. 1999).

Mass peristaltic events are of relatively long duration (9.7–2.8 s in the rabbit) and propagate rapidly (8–21 mm/s in the rabbit). The leading edge of the contraction has a step-like appearance in the rabbit colon (Lentle et al. 2008), which probably results from the summation of mass peristalsis with myogenically generated fast phasic contractions, the former propagating more slowly than the latter. The pattern of contraction and recovery during mass peristalses is temporally and spatially asymmetric on spatiotemporal maps, the interval between the onset of constriction and subsequent recovery varying along the haustrated colon (Lentle et al. 2008). In particular, the return of the lumen to a distended state following its constriction during the mass peristaltic event occurs more slowly in the proximal than the distal haustrated colon. The constriction at the leading edge of mass peristalses, generated by the concerted action of the longitudinal and circular muscles, promptly propels the contents distally. The return of the lumen to its original dimensions following the subsequent relaxation of these muscles depends upon the retrograde flow of the distally displaced material. Any watery liquid and contained fine particulate matter that has been expressed from digesta during mass peristalsis will flow more readily into a relaxed proximal segment of the haustrated colon than will the compacted mass of digesta from which it has been expressed. Hence the fluid and fine particulate phase may be selectively refluxed following mass peristalsis.

9.2.1.2 Fast Phasic Contractions

The frequency of ongoing fast phasic contractions appears to vary spontaneously between several discrete values that are similar to those of colonic electrical activity reported by other workers (Pescatori et al. 1980). They occur at intervals of around 2.3, 3.5, 5.0 and 7.5 s in the rabbit (Lentle et al. 2008), which approximate ratios of 1:2:3:4. This variation in frequency is suggestive of the operation of a high-frequency pacemaker coupled with a variation in the refractory period of the responding smooth muscle. It is probable that fast phasic activity is present in the colon of other species though not identifiable without the use of high-definition spatiotemporal mapping techniques. This activity may aid in reducing the apparent viscosity of the contents, particularly at the periphery of the digesta plug, which may promote the plug flow of viscous digesta (Lentle et al. 2007). It may also constitute the means by which muscular tone (Ford et al. 1995), the distensibility and the capacity of colonic components are altered. Hence the contractile component of Hill's model (Hill 1970), which is likely to describe the distribution of stress in the tissue of the gut wall (Gregersen 2003), may result from 'tetanic' rather than specific tone (Mandrek and Golenhofen 1990), the component high-frequency contractions only being evident in high-resolution spatiotemporal maps.

9.2.1.3 Haustral Progression

A body of evidence indicates the colon of a number of species is capable of a third type of motility characterised by slow lengthwise progression of haustral boundaries. Such a progression was described in the human colon as '…annular constrictions of the barium mass, which appeared to originate as interhaustral folds and advanced more or less steadily along the bowel in either direction like waves', a process termed 'interhaustral progression' by the researcher (Ritchie 1968a). A similar pattern of slow haustral movement with incomplete lumen occlusion was observed in high-resolution spatiotemporal maps of the ex vivo rabbit colon (Lentle et al. 2008). The rate of progression (0.13 ± 0.02 mm/s) and frequency (0.53 cycles/min) determined by these workers was similar to those of patterns seen on fluoroscopy and in the outputs of pressure transducers recorded in vivo in the same species (Ehrlein et al. 1983; Ehrlein and Ruoff 1982).

Slow progression of a highly localised area of radial constriction may provide the means of transporting viscous or semisolid digesta without generating high pressure. The condensation of colonic longitudinal muscle into taenial bands reduces the thickness and increases the compliance of the wall in the domains between taeniae. The latter allows a deep localised constriction to be formed while maintaining distension and compliance in adjacent regions. The resistance to onflow offered by the residual lumen space between three taeniae (Langer and Takács 2004) may be lower than the more circular space generated by a small intestinal peristaltic contraction profile. Moreover, a contractile pattern that induces movement of digesta without undue compression of the contents may reduce collapse of the particulate matrix and thus preserve the ability of intrahaustral ripples to induce expression and permeation of the fluid phase through the matrix (see below). In the distal parts of the colon, where taenial modification of lumen geometry does not occur, haustral progression may similarly reduce the pressures generated during the propulsion of more solid digesta. However, it is noteworthy that in Western populations diverticulosis, an out-pouching of the colonic wall, which may result from lumen overpressure, commonly commences in the more distal and less haustrated colon where wall compliance, tensile strength and burst strength are lower (Mimura et al. 2002).

9.2.1.4 Intrahaustral Ripple Contractions

A fine short-lived ripple motility occurs within the confines of haustra in the rabbit (Lentle et al. 2008). This motility pattern has similar characteristics to the 'anti-peristalsis' reported in the human colon by earlier workers (Cannon 1902), given that these workers had no means of distinguishing between haustral boundaries and intrahaustral folds. A similar ripple motility pattern has been reported in the guinea pig colon (D'Antona et al. 2001) and in the rabbit (Ehrlein and Ruoff 1982), although the ripples in the rabbit colon were sustained while those in the guinea pig occurred more sporadically. It has been postulated that the concerted action of ripples within haustra may serve to propel liquid phase aborad. No coordination was evident between individual colonic ripples within a haustrum in ex vivo preparations of rabbit colon (Lentle et al. 2008). However, these workers did note that ripple contractions exhibited greater coordination during periods when haustral progression was abolished for a short period, notably after a mass peristaltic event. Although, even under these conditions, no consistent overall direction of propagation was established (Lentle et al. 2008).

These findings and the likelihood that ripple contractions are myogenic, as they are not inhibited by agents that interfere with neural conduction (Lentle et al. 2008), shed some doubt on the single report that coordination of colonic ripple contractions occurs in vivo (Ehrlein et al. 1982). They also shed doubt on the likelihood that the selective retention of small nutrient-dense particle by the rabbit colon (Björnhag 1981, 1987) results from coordinated ripple contractions.

A similar situation may occur in humans, roentgenographic studies showing that 'systolic' contractions of individual 'haustra' cause the contents to be displaced into adjacent haustra, barium being ejected in both directions equally (Ritchie 1968a, b). The same study reported that successive 'haustral' contractions propelled colonic contents either orad or aborad. However, it is not possible to tell from the published radiographs whether the contracting elements were haustra (Lentle et al. 2008) or individual ripples, as seems more likely.

It is possible that there is microscopic morphological specialisation of those elements of circular muscle that contribute to the boundaries of ripples and haustra. The persistence and relative position of static radial folds following the addition of verapamil indicate that certain locations along the length of the colonic segment show a greater inherent propensity to form folds (Lentle et al. 2008). Histological studies similarly report that circular muscles are thicker at folds than within sacculations (Cormack et al. 1987). Hence there may be a number of populations of smooth muscle cells in the sacculated colon that have differing functional roles: those mediating haustral progression, which may arise from the continuation of taenial longitudinal muscle bundles into the circular muscle layer (Langer 1988); and those that contract in response to local slow waves to produce ripple contractions (Ross and Pawlina 2006).

9.3 The Stomach

The form of the mammalian stomach differs from that of the other components of the intestinal tract in that it generally departs from a simple tubular configuration (Langer 1988). The form varies between species principally in regard to diet (Langer 1988; Stevens and Hume 1995). Its morphology ranges from a simple uniloculate structure, in which there is regional specialisation, to complex plurioculate structures, in which there is compartmental specialisation (Stevens and Hume 1995).

The uniloculate stomach is sacculate in configuration comprising a series of subsidiary regions of differing structure and function (Schulze 2006). Gastric function is complicated by the integration of (periodic) accommodation in the proximal compartments with ongoing trituration, digestion and selective passage of finer materials in the distal compartments. Hence its function is not accurately represented as that of a simple continuously stirred tank reactor (Penry and Jumars 1986).

The proximally situated fundus is thought to accommodate incoming ingesta by receptive relaxation (Blat et al. 2001), and subsequently to promote the onflow of digesta by reducing its volume (Hausken et al. 2002; Pal et al. 2004; Paterson et al. 2000). This onflow is periodically facilitated by antral contraction waves (Camilleri et al. 1985; Malbert and Mathis 1994; Miller et al. 1981), which promote flow towards the pylorus and lower the pressure immediately distal to the fundus.

The wall of the proximal stomach contains an obliquely oriented layer of smooth muscle, which may aid in the control of dynamic compliance and the contained volume (Gregersen 2003). The antrum and pyloric canal are thought to triturate and mix digesta by retropulsion (Camilleri et al. 1985; Faas et al. 2001; Indireshkumar et al. 2000; Marciani et al. 2001a), by direct radial compression (Marciani et al. 2001a), and by shear resulting from the sloping configuration of the walls (Gregersen 2003). The pyloric sphincter is thought to restrict onflow of digesta into the proximal small intestine (Kwiatek et al. 2006; Malbert and Ruckebusch 1991; Wang et al. 2005).

The combined actions of these various compartments result in the early onflow of liquid phase and the more tardy onflow of elements of the solid phase of digesta (Kunz et al. 2005; Maes et al. 1998). Thus larger particulate material is retained until it is adequately triturated, diluted (Meyer 1991) and reduced in size, this action having been termed ‘gastric sieving’ (Hinder and Kelly 1977; Marciani et al. 2001c).

A number of morphological modifications to the uniloculate stomach in species of differing dietary habit suggest that there has been evolutionary adaptation to restrict flow between the component regions. For instance, in the uniloculate stomach of New World *Cricetinae,* the antrum is rotated to a more vertical orientation with respect to the proximal stomach and a septate division is formed between the proximal and distal stomach. These modifications may serve to sequester ingested food boluses proximally and to avoid their dispersal by the material that has been retropulsed from the gastric antrum, a strategy that would enable more complete digestion of starches in their granivorous diet by salivary amylases (Carleton 1973).

9.3.1 The Timing and Volume of Food Intake: Meals and Gastric Function

For maximal efficiency in the gastric processing of food, the accommodative action of the proximal compartments must be integrated with the triturative and digestive actions of the distal compartments. It is therefore important to consider whether the volume of a meal is restricted so as to allow the functions of the distal compartments to continue rather than being disrupted by complete filling of the entire gastric lumen with food boluses. A body of work indicates that gastric fill can induce satiety via vagal feedback from mechanoreceptors in the gastric wall (Powley and Phillips 2004; Smith 1998), i.e. gastric or volumetric satiety. However, the work does not indicate whether such satiety is induced as gastric fill approaches the limit of its function of volumetric retention or as it approaches the limit of its ability to efficiently mix and digest its contents.

If the principal means of controlling meal size is by satiety, gastric fill may be limited to allow the continuing efficient function of the more distal compartment. Conversely, if the principal means of controlling meal size is by hunger arising from energy deficit, there may on occasion be overfill and temporary disruption of the efficient function of the more distal compartments of the stomach. If the size of a meal were regulated principally in relation to satiety then we may expect to see a strong correlation of meal size with the interval following the meal, i.e. postprandial correlation. Alternatively, if the size of a meal were regulated principally in relation to hunger then we may expect a stronger correlation of meal size with the time since the previous meal, i.e. preprandial correlation.

It is known that the operation of short- and medium-term physiological responses based on satiety or hunger result in the temporal grouping of feeding events into meals (Le Magnen 1992). However, the degree of postprandial and preprandial correlation (Castonguay et al. 1986; Collier et al. 1999) seems to be vulnerable to imposed scheduling of the meal. For instance, when free-feeding laboratory rats are restricted to five meals per day, meal size becomes temporarily correlated with the duration of the preprandial interval but subsequently reverts to a postprandial correlation as the animals become more used to the new feeding routine (de Castro 1988). Hence in monogastric animals, it appears that the external scheduling of a meal could on occasion lead to gastric overfill. More recent work shows a higher level of complexity in that 'snacks' as well as meals constitute a significant part of the human diet but are not consumed in a sufficient quantity to ameliorate the onset of hunger during the time to the next meal (Bellisle et al. 2000). These findings led the workers to conclude that the internal signals associated with hunger and satiety do not define eating patterns in humans in a simple linear causal relationship (Bellisle et al. 2000).

9.3.2 Gastric Contractile Activity

Recent evidence suggests that the manner in which the function of the various compartments of the human stomach is integrated may be influenced by the physical properties of the contained digesta. For example, the addition of viscogenic agents delays gastric emptying, induces local antral distension and promotes gastric satiety (Marciani et al. 2001b; Powley and Phillips 2004).

Evaluation of the effects of the physical properties of digesta on contractile activity in the various gastric compartments is rendered complex in vivo by ongoing dilution with gastric secretions (Marciani et al. 2001b), feedback effects of various nutrients (Sakaguchi et al. 1994) in conjunction with feedback regulation via gastro-entero-pancreatic hormones (Lu and Owyang 1995; McTigue and Rogers 1995; Schirra et al. 2000; Takahashi and Owyang 1999) and elements of the autonomic nervous system (Lefebvre et al. 2005). Moreover, assessment has been complicated by experimental difficulties, e.g. the prokinetic effects of intragastric measuring devices such as barostats (Mundt et al. 2002; Ropert et al. 1993), and the atypical positioning of the subject that is required for some external measuring devices, such as MRI (Kwiatek et al. 2006), which may disrupt the normal sedimentation of digesta within the human gastric lumen.

The use of ex vivo preparations of the stomach has enabled many of these confounding effects to be eliminated, thereby allowing for finer resolution and quantification of inherent wall movements using spatiotemporal mapping techniques (Berthoud et al. 2002; Hennig et al. 1997; Lentle et al. 2010). The general form of the motor activity in the isolated stomach has been described as 'virtually identical to that in vivo despite the absence of autonomic input' (Schulze-Delrieu et al. 1998). Hence such preparations are useful in describing the intrinsic motility patterns engendered by myogenic and intrinsic neuronal mechanisms.

9.3.2.1 Antrocorporal Contraction Waves

Antrocorporal contractions generally commence on the greater curvature of the proximal corpus and propagate distally, increasing in amplitude and extending to involve the lesser curvature just distal to the oesophageal opening. Propagation ceases just short of the pyloric sphincter, possibly due to local absence of the elements necessary for their propagation (Wang et al. 2005). The frequency of antral contractions is such that between two and four contractions are traversing the stomach at any one time.

Antrocorporal contractions appear on spatiotemporal maps as regularly spaced sloping lines (Fig. 9.1). The amplitude of contraction, evidenced by the relative intensity of shading of the lines, is noticeably smaller on the lesser than the greater curvature. Occlusion of the antral lumen, which results from the synchronous contraction of the greater and lesser curvature is generally incomplete with occlusions of approximately 60% of the cross-sectional area being found in both the human stomach (Marciani et al. 2001c) and the ex vivo stomach of the rat (Lentle et al. 2010). The point of maximum occlusion occurs just proximal to the point on the pylorus at which the contractions terminate.

In some ex vivo preparations, the velocity with which antrocorporal contractions propagate increases in the antrum relative to that in the corpus being 2.8 ± 0.09 mm/s in the ex vivo guinea pig antrum compared with 1.3 ± 0.05 mm/s in the corpus

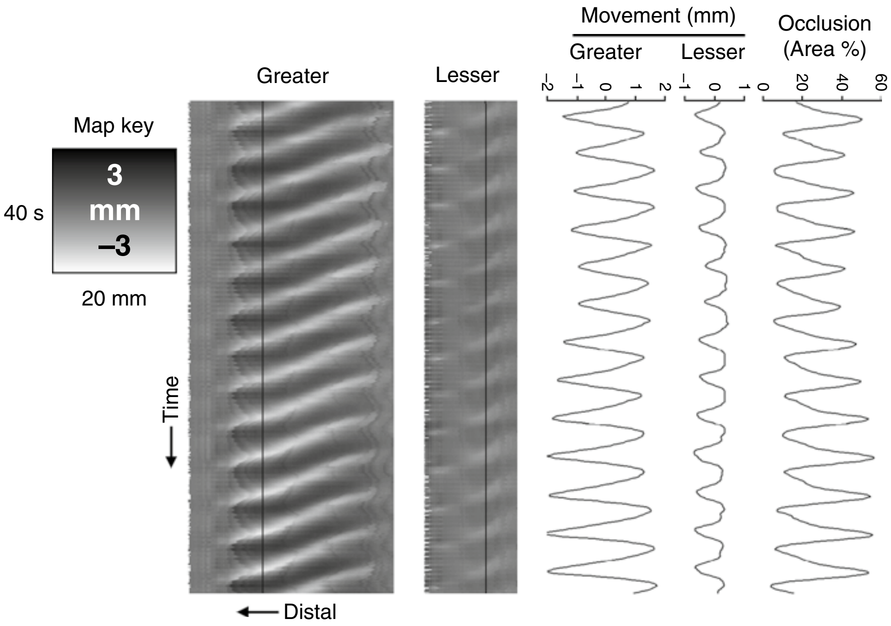


Fig. 9.1 Spatiotemporal maps of the greater and lesser curvatures of a rat stomach that was perfused with 4 ml of 1.5% guar gum solution. The line plots on the right correspond to amplitudes and occlusion calculated at the position shown by the vertical black lines shown on the maps. (Lentle et al. 2010)

(Hennig et al. 1997), and 1.85 ± 0.07 mm/s in the ex vivo rat antrum compared to 0.94 ± 0.17 mm/s in the corpus (Lentle et al. 2010). Similarly the velocity of propagation of slow waves is reported to increase from 5 ± 2 mm/s in the corpus of the in vivo canine stomach to 15 ± 6 mm/s in the antrum (Lammers et al. 2009).

The volume of fluid pumped towards the antrum is also dependent on the geometry of the antral wave (Pal et al. 2007). Current knowledge of antral wave geometry is limited due to the time and spatial constraints of in vivo methods, such as MRI (Schwizer et al. 1996). Spatiotemporal mapping of the ex vivo rat stomach shows that the form of antrocorporal contractions is approximately symmetrical though of greater amplitude in the greater than in the lesser curvature (Fig. 9.1). Similarly, the pattern in the rise and fall in strain magnitude is symmetrical in the human antrum 2 cm proximal to the pylorus (Ahmed et al. 2009). Thus these geometries differ from those of propulsive events in the small intestine (Lentle et al. 2007; Schulze-Delrieu 1999), in the oesophagus (Mittal et al. 2005), and in the colon (Lentle et al. 2008). The difference in form of the antrocorporal phasic contraction from that in the small intestine likely results from differences in the firing sequence of the layers of smooth muscle. Contraction of circular and longitudinal muscle layers occurs simultaneously at the same site during antrocorporal contraction (Sarna 1993), while there are differences in the sites and timing of longitudinal and circular contraction during small intestinal peristalsis (see Chap. 8). The effects of such differences in contractile form may differ correspondingly in that material displaced by the advancing wave of gastric constriction may not be so readily accommodated in the area immediately distal to the site of contraction. Hence synchronous firing would augment the overall speed of onflow. Indeed, work by Gregersen et al. (2006) shows that distension engenders distal phasic antral contractions that are accompanied by longer lasting increases in antral tone and indicates that the distensibility (Gregersen and Kassab 1996) of the antral wall adjacent to the site of phasic antral contraction may be adjusted to promote onflow.

Human in vivo studies using MRI show that the frequency and speed of propagation of antrocorporal contractions does not vary with the physical properties of the meal (liquid, mixed liquid/solid or mixed viscous/solid) (Marciani et al. 2001c). Similarly, neither the frequency, amplitude nor velocity of antrocorporal contractions differs when pseudoplastic (1.5% guar gum) rather than watery fluid is perfused through the ex vivo rat stomach (Lentle et al. 2010). Conversely, a study of antrocorporal contractions in dogs using a series of (pseudoplastic) slurries of potato granules showed that the amplitude of antrocorporal contractions declined with increase in apparent viscosity (Prove and Ehrlein 1982). However, the latter findings are likely to have been complicated by feedback effects from the nutrient content of the perfusate.

While the bulk of evidence suggests that the characteristics of antrocorporal contractions are not affected by change in the viscosity of pseudoplastic or Newtonian fluids, it appears that they do change when the stomach is perfused with fluids that generate patterns of strain which are not normally encountered in the stomach wall. Thus the site of origin, overall speed, frequency and direction of propagation of antrocorporal waves changes significantly when the ex vivo rat stomach is perfused with material of similar apparent viscosity to 1.5% guar gum but which exhibits shear thickening and extensional flow (Lentle et al. 2010).

Antral tone appears to vary inversely with corporal tone. A number of hormonal agents act to increase corporal but not antral tone (Morgan and Szurszewski 1980). Hence a timely neuronal and hormonal stimulus may augment a general gradient in pressure between corpus and antrum, which aids progression of material into the distal stomach (Lacy and Weiser 2005). The mechanism of this progression is discussed in more detail below. It is noteworthy that antral distension does not appear to influence basal antral tone (Caldarella et al. 2003).

9.3.2.2 Fundal Tone

The fundus is thought to influence the flow of digesta in the stomach by changes in its tone so as to influence the distensibility of its walls. A lowering of its tone contributes to the accommodation reflex, which follows consumption of a meal (Cannon 1898), and an increase in its tone promotes onflow into the distal stomach and beyond (Azpiroz and Malagelada 1985; Kelly 1980; Lind et al. 1961).

The volume of the fundus increases to a greater extent than that of the antrum and corpus when the isolated guinea pig stomach is perfused by watery material

(Hennig et al. 1997), and when the ex vivo rat stomach is perfused by viscous material (Lentle et al. 2010). Evidence suggests that in the ex vivo stomach, fundic accommodation results in part from the redistribution of freshly ingested material from the corpus to the fundus. Specifically, the perfusion following paralysis of gastric smooth muscle with verapamil causes most of the consequent increase in volume to be restricted to the fundus (Lentle et al. 2010), while perfusion following the administration of tetrodotoxin (a neural inhibitor) reduces fundal expansion but augments the expansion of the antrum and corpus (Hennig et al. 1997). Together these findings, along with the observation that the fundus decreases slightly in volume on cessation of perfusion (Hennig et al. 1997), suggest that the relative distribution of incoming ingesta between the fundus and corpus is controlled by the balance of resting tone in the fundus and corpus, and that changes in the volume of the latter are induced by local neural reflexes, i.e. do not require spinal circuitry (Balchum and Weaver 1943; Cervero 1991; Morrison 1977). While fundal contractions do not occur in the ex vivo stomach, they are reported in vivo in innervated fundal pouches (Lind et al. 1961) and when pressure is monitored by a fundal barostat (Nguyen et al. 2007), and hence may be mediated by extrinsic neuronal pathways.

A body of evidence indicates that fundal responses are more sophisticated in vivo. For instance, oil (Edelbroek et al. 1992) and solid foods (Collins et al. 1988) are retained in the forestomach for longer periods than watery fluids (Guerin et al. 2001), viscous foods are retained in the forestomachs of pigs for longer periods than less viscous foods (Guerin et al. 2001), and addition of fat to a meal causes a greater proportion of material to flow back from the distal to the proximal stomach (Houghton et al. 1990). There are indications that these more sophisticated responses involve neuronal and hormonal feedback. Infusion of nutrients directly into the duodenum initiates a sustained increase in the volume of the proximal gastric compartment (Nguyen et al. 2007), while the intravenous administration of ghrelin acts to reduce fundic volume (Ang et al. 2009). There is also evidence of feedforward coordination between fundic and duodenal activity. Distension of the fundus initiates both antral and duodenal motility in human subjects (Rao et al. 2002). The walls of the fundus and antrum are thought to contain stretch and tension mechanoreceptors (Novitol et al. 1995; Piessevaux et al. 2001) but it not known which type of receptor is responsible for inducing the changes in motility.

The contractile activity and tone of the fundus are known to be modulated by the vagus. Selective vagotomy of the proximal stomach hastens the emptying of liquids but has no effect on the emptying of solids (Wilbur and Kelly 1973). Vagotomy is known to bring about a short-term decrease in the accommodative capacity of the fundus in the rat (Takahashi and Owyang 1997), dog (Wilbur and Kelly 1973) and the ferret (Andrews et al. 1980), but receptive relaxation appears to eventually be reinstated, possibly as a result of adaptive changes in the gastric myenteric plexus (Takahashi and Owyang 1997).

The mechanisms that influence fundal relaxation, notably whether gastric accommodation results principally from an 'anticipatory' or from a distension-induced reflex, and the timing of any subsequent return of fundal tone and pressure to promote onflow have not been fully elucidated. Earlier work measuring the magnitude of change in fundal tone and capacity was carried out using a barostat (Azpiroz and Malagelada 1985). This led to the identification of a general increase in fundal capacity immediately following a meal, which was described as the accommodation reflex (De Schepper et al. 2004). More recent MRI studies indicate that the gastric accommodation reflex appears to be influenced by the presence of the barostat, and may even have been induced by the device (de Zwart et al. 2007). This and other work, which compared results from Single photon emission computed tomography (SPECT) and ultrasonographic evaluations of meal-induced change in fundal volume with those obtained with the barostat, failed to detect any accommodation response over and above that of the volume of the ingested meal (Mundt et al. 2002; Van den Elzen et al. 2003). Hence there may not be a period during which the volume increase of accommodation exceeds that of the incoming meal nor any time delay for the extinction of the gastric reflex and/or the remission of excess volumetric expansion before tonal change directly increases lumen pressure. This would eliminate any reflex in which an increase in gastric capacity over and above the volume of the meal would act to preserve the efficient function of more distal compartments of the stomach.

There is evidence that the liquid and solid components of a meal are differently dispersed within the stomach. Human scintigraphic studies show that the liquid component of a meal is rapidly dispersed through the stomach with a lag time as short as 1 min, while the solid component can be retained in the proximal stomach for as long as 95 min (Collins et al. 1991). Other work (see below) suggests that there is prompt commencement of emptying of the labelled solid phase of a meal from the proximal to the distal stomach so that markers moved promptly from the former with retention decreasing as a power exponential to increase in the latter (Guerin et al. 2001).

9.3.3 Flow Within the Stomach

9.3.3.1 Early Postprandial Flow

Liquid digesta travels rapidly distad in the human stomach following a relatively narrow, curved, dorsally situated path, through the 'magenstrasse' (Eisenberg 2002), an anatomical region adjacent to the lesser curve of the stomach in which parallel folds of mucosa (rugae) run lengthwise. Dorsal structural and physiological adaptations that provide a path for the passage of liquid digesta, e.g. a dorsal groove, have been described in the pluriloculate stomachs of ruminants and macropods (Stevens and Hume 1995). However, flow within the 'magenstrasse' appears to result from the hydrodynamic consequences of its position rather than any physiological adaptation. Thus freshly ingested liquid enters the stomach just proximal to the site of origin of antrocorporal contraction waves, which each generate a low-pressure region on their trailing edge that preferentially 'pulls' the liquid (low viscosity) component of chyme or ingesta aborad. The existence of such a mechanism is supported by

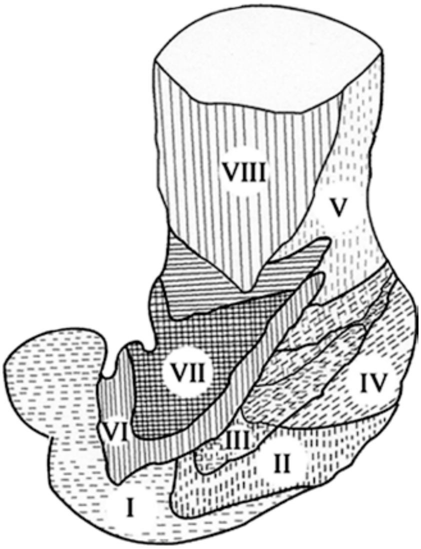


Fig. 9.2 Layering of food boluses in the human stomach. Food boluses traverse the cardia and progress distally down the lesser curvature, with sequential boluses stacking up, each with a V-shaped configuration. Earlier boluses are displaced towards the greater curvature where they may spread orad. Reconstruction by Groedel (1924) is based on serial radiographic observations. (Schulze 2006)

findings that flow in the 'magenstrasse' does not occur in subjects with low levels of gastric motility (Jefferson 1915).

The fate of solid digesta differs from that of liquid digesta. In human subjects, semisolid boluses become sequentially layered from distal to proximal in the stomach lumen with material that had been ingested earlier being situated closer to the antrum on the greater curvature and the more recently ingested material being situated closer to the lesser curvature and the fundus (Groedel 1924; Toyama and Blickman 1971) (Fig. 9.2). This disposition serves to underscore the orderly transit of a sequence of boluses through the gastric lumen and the persistence of the integrity of the boluses for a significant period of time after entering the stomach, thereby allowing time for in situ digestion by salivary amylase and lipase (Marciani et al. 2001c).

Such sequential stacking could occur passively during postprandial stasis or from pressure generated by fundal contractile activity, but it is unlikely to persist in the face of continuing antrocorporal contractions. It is to be expected that the order of 'stacking' would be rapidly disrupted by the differing amplitude of antrocorporal contractions along the lesser than the greater curvature (Carlson et al. 1966; Keinke and Ehrlein 1983; Lentle et al. 2010; Schulze-Delrieu and Herman 1998) and by the forcible retropulsion of gastric contents from a closed pylorus (Berstad et al. 1994; Keinke and Ehrlein 1983), as indeed is suggested by the V-shaped outline of the dorsal boluses and proximally displaced portions of the early boluses in Groedel's original diagrams. Hence the early postprandial progress of boluses towards the antrum may reflect a combination of simple progressive displacement and pressure changes generated by regional differences in gastric tone (Schulze 2006), the movement being lubricated by gastric secretions accumulating between the boluses and the gastric mucosa (Marciani et al. 2001c).

9.3.3.2 Mixing Patterns

Sequences of antrocorporal contractions acting against a closed pylorus are likely to produce a net distal movement of material at the perimeter of the lumen and a net proximal movement of material at the centre of the lumen (Pal et al. 2004). This would result in the establishment of a vortical pattern of flow and generate mixing in the distal compartment of the stomach. Semisolid boluses tend to persist in the fundus and to maintain their integrity for longer with gastric secretions slowly permeating and eroding their periphery (Marciani et al. 2001b). The same study showed that the eroded and dispersed peripheral components of the boluses exited the stomach earlier than the material that remained in the bolus.

Computer simulations have also shown smaller vortices may be generated at the gut wall in the space between successive antrocorporal contractions (Pal et al. 2004). While the establishment of such vortices would be useful in promoting further mixing, they have not been seen in vivo (Schulze 2006). Simulations have also indicated that ingested material rapidly moves along an axially situated path from the fundus to the distal stomach, confusingly also termed the ‘magenstrasse’ by the authors (Pal et al. 2007). Unlike the more dorsally situated path normally associated with the magenstrasse (Eisenberg 2002), this axial path has not been observed in practice. It is possible that some of the results of this model are artefactual as the model did not incorporate boundary conditions that allowed for the effects of fundal expansion and transpyloric flow during early antrocorporal contractions. Further, the results may reflect shortcomings in the rheological description of gastric digesta in the model; in particular, gastric digesta is a heterogeneous mixture of viscoelastic boluses in a non-Newtonian fluid.

9.3.4 Trituration

Phasic contractions of the antrum and pylorus are thought to contribute to the breakdown of food particles and to have a ‘sieving’ action, which brings about the selective gastric retention of any particles larger than 1 mm (Schulze 2006). Particles can be broken down in the antrum either by their direct compression during radial constriction of the walls of the antrum (Marciani et al. 2001a) or by shear forces generated during the forcible retropulsion of fluid digesta that has been pumped distally by the antral contractions towards a closed pylorus (Camilleri et al. 1985; Faas et al. 2001; Gregersen 2003; Indireshkumar et al. 2000; Marciani et al. 2001a). It has also been postulated that the bolus itself is subjected to shear when it is forced into a narrow section of the pyloric canal (Gregersen 2003).

9.3.4.1 Forcible Retropulsion

The rate of pumping of fluid towards (and away from) the pylorus, and hence the magnitude of shear, will be a function of the speed of propagation, the amplitude, the frequency and the form of antrocorporal contractions. The maximum shear rate calculated on a basis of volume displacement and the minimum diameter of the antral lumen, as estimated from spatiotemporal maps of an ex vivo preparation of a rat stomach, is around 0.7 s^{-1} (Lentle et al. 2010). It is noteworthy that some antrocorporal contractions propagate more rapidly in the antrum, where the maximum shear rates occur, than in the corpus. Calculations using models of the human stomach (Pal et al. 2004) based on echoplanar MRI slices indicate that the velocity of the retropulsive jet is around 7.5 mm/s and the maximum surface shear stress received by food particles in human subjects is around 10–30 Pa. Thus it appears that only moderate shear forces are generated during antral retropulsion.

In ex vivo preparations of the rat stomach, the frequency of antrocorporal contraction waves decreases with increase in gastric fill, while their amplitude increases (Lentle et al. 2010). However, the mean velocity of propagation of antrocorporal contractions does not vary with gastric fill. Taken overall, these findings suggest that shear rate does not change appreciably with gastric fill in the isolated stomach. Conversely, local distension of the human distal antrum in vivo is reported to increase the frequency of antral contractions and the level of antral tone (Gregersen 2003; Gregersen et al. 2006).

If the generation of shear by the forcible retropulsion of digesta is important in particle breakdown, then the force generated by antrocorporal contraction should increase postprandially, given that the apparent viscosity of stomach contents will be higher at this time (Groedel 1924). Radial strain is known to increase during the intermeal period of migrating motor complex (MMC) activity, i.e. there is greater force of antral contraction during phase III than during the early phase I period of the MMC cycle, but there is no overall significant difference in the forces generated by these contractions from those during the postprandial period (Ahmed et al. 2009). It remains to be determined whether changes in the physical properties of gastric chyme directly influence the characteristics of antral contractions in vivo. The apparent viscosity of the perfusate does not appear to influence the characteristics of antral waves in ex vivo preparations provided it is a Newtonian or pseudoplastic fluid. Thus neither the frequency, amplitude nor velocity of antral contractions differs when 1.5% guar gum solution is perfused rather than saline (Lentle et al. 2010). Hence in ex vivo rat stomach, there appears to be no compensatory increase in the force applied with increase in the apparent viscosity of the contents.

9.3.4.2 Direct Compression

The magnitude of the force that is directly applied by the antral walls has been reported at around 0.2 N in human subjects using a balloon catheter (Camilleri and Prather 1994; Vassallo et al. 1992). However, the presence of the device may have stimulated the onset of more forcible contractions. Other workers using a range of tablets containing a marker drug with an outer Teflon layer of known fracture strength estimated that fracture forces were around 1.5 N under fasting conditions and 1.89 N under fed conditions, the former presumably reflecting phase II or phase

III activity (Kamba et al. 2000; Kamba et al. 2001, 2002). A more conservative approach comprising the feeding of agar gel beads of known strength shows the postprandial antral contractile force is around 0.65 N (Marciani et al. 2001a). Significant lumps of alginate gel with a fracture strength around 0.7–1.2 N persist in the human gastric lumen for 125 min after ingestion (Hoad et al. 2004), while the half residence time of agar beads of strength below 0.65 N is below 30 min (Marciani et al. 2001a). These survival times indicate that the direct application of force is relatively inefficient in breaking down heterogeneous lumps of gel.

To secure breakage of all particles regardless of size, antral contractions should proceed to complete occlusion. While the amplitudes of antral contractions increase as they progress distally (Kwiatek et al. 2006; Schulze-Delrieu et al. 1998), they do not completely occlude the lumen either in the ex vivo rat stomach (Lentle et al. 2010) or cat stomach (Schulze-Delrieu et al. 1998). However, complete occlusion is reported in the terminal 4 cm of the human gastric antrum but occurs relatively infrequently during the early intermeal interval (5.2% of contractions during phase II) and more commonly just prior to eating (52% of contractions during phase III) (Ahmed et al. 2009). Taken together, these findings indicate that occlusive antral contractions may function principally to break down and clear resistant particulate residues from the antral lumen during the intermeal interval and may not play a significant part in postprandial trituration.

9.3.4.3 Gastric Sieving

A body of evidence shows that the stomach selectively retains course particles and allows onflow of finer particles, an action known as gastric sieving (Meyer et al. 1979). For example, greater proportions of coarse than fine bran particles (McIntyre et al. 1997) and greater proportions of fine than very fine resin particles (Burton et al. 1995) are retained in the human stomach. This action retains particles greater than 1.4 mm in the dog (Becker and Kelly 1983) and particles greater than 1 mm in human subjects (Pera et al. 2002).

Gastric sieving may result from the action of any one of the regions of the stomach or from outcomes of their coordinated action. The preferential retention and digestion of solid material in the fundus and proximal corpus (Collins et al. 1988) has been discussed earlier. In addition, antropyloric contractions that pump fluid distad through the open pylorus at some stages of antrocorporal progression may carry floating or neutrally buoyant (Meyer et al. 1986) particles with it, in effect decanting them from larger denser non-buoyant particles in the lower portion of the greater curve, i.e. the gastric sinus (Brown et al. 1993; Schulze 2006). However, buoyant fat droplets or solid particles containing a high proportion of fat that float on the surface of the gastric contents may be skimmed off by the incisural fold, which projects downwards from the lesser curvature (Schulze 2006). It is possible that this decanting process is aided by the greater amplitude of antral waves around the greater than the lesser curvature (Lentle et al. 2010) causing greater volumes of liquid to be presented to the pylorus from the lower portion of the stomach. The operation of this mechanism depends on posture (Horowitz et al. 1993), and hence does not seem tenable in quadrupeds.

Another possible mechanism of retention of larger particles is by filtration, i.e. the sequestration of course particles by the formation and ongoing filtering action of a particulate matrix (Ramkumar and Schulze 2005). This could occur if a mat of entangled larger particles formed within the pyloric antrum and acted as a basis of filtration or permeation, i.e. a filter media (Schulze 2006). However, it is difficult to envisage at which point during the MMC cycle that the formation of such a mat is likely to be beneficial. While a mat may form during those phases of the MMC cycle when antrocorporal contractions are coordinated with pyloric opening and transpyloric flow (Savoye-Collet et al. 2003; Savoye et al. 2003), this is thought to be the time when housekeeping contractions act to clear the stomach of all particulate residues rather than acting as a filter. Again, in the fed state, pyloric opening occurs at times when an antrocorporal wave is traversing the body and proximal antrum but pyloric constriction occurs as the wave approaches the pylorus (Indireshkumar et al. 2000). Hence the pylorus will close at the time when the antral contraction is most likely to force chyme into the constricting portion of the antrum (Pal et al. 2007) to promote mat formation.

A final possible mechanism, which would operate successfully in the fed state, is that the jet of chyme that is retropulsed from the closed pylorus acts as a virtual impactor (Lentle and Janssen 2008), i.e. a mechanism that separates larger and denser particles from lighter smaller particles by imparting sufficient inertia to them to propel them a greater distance orad from the closed pylorus so that they are less liable to exit the stomach during times when the pylorus is open. Such a mechanism has been alluded to by earlier workers (Prove and Ehrlein 1982). The formation of an axial retropulsive jet is well established (Schulze-Delrieu et al. 1996b; Schulze-Delrieu et al. 1998) and, there is some indirect evidence to support the operation of such a mechanism. The stomach of the rat has been reported to retain a greater proportion of denser particles than of less dense particles of the same size (Tuleu et al. 1999).

9.3.5 Onflow from the Stomach

The emptying of the chyme from the stomach is an outcome of the actions of its various components but does not represent a simple sum of them, rather it is primarily governed by the level of fundic accommodation, i.e. wall tension, and the opening of the pylorus and its relationship to the distal progression of the antrocorporal waves (Keinke and Ehrlein 1983). Both vary according to the fed or fasting state as has been discussed earlier. The passage of particulate matter is also governed by the phase of MMC cycle. Thus in the stomach of the dog, the passage of particulate material of sizes greater than 1 mm occurs more readily during phases II and III of the cycle than in the postprandial period. As a result, 90% of inert beads of sizes ranging from 0.5–6.4 mm transited the pylorus after the passage of a single MMC (Gruber et al. 2006).

The magnitude of the contribution of antrocorporal waves to gastric emptying in the fed state is questionable. Medications that increase antral contraction fail to influence gastric emptying (Talley 2003), and MRI studies show that pulsatile emptying is increased but not absolutely correlated with antral contraction waves (Kwiatek et al. 2006) (see also Sect. 7.3.3). Hence the regulation of onflow, while dependent upon fundal and corporal tone, is largely in line with the early hypothesis that the pylorus is 'the keeper of the gate' (Cannon 1898).

9.3.5.1 Mathematical Descriptions

The exponential function given below is known to be generally useful in the analysis of biphasic curves (Siegel 1985; Siegel et al. 1985):

$$y = 1 - \left(1 - e^{-kt}\right)^{b}$$

where y is the fractional solid retention at time t, k is an exponential emptying rate and constant b is the extrapolated y-intercept from the terminal portion of the curve. A lag time t_{lag} can also be calculated from

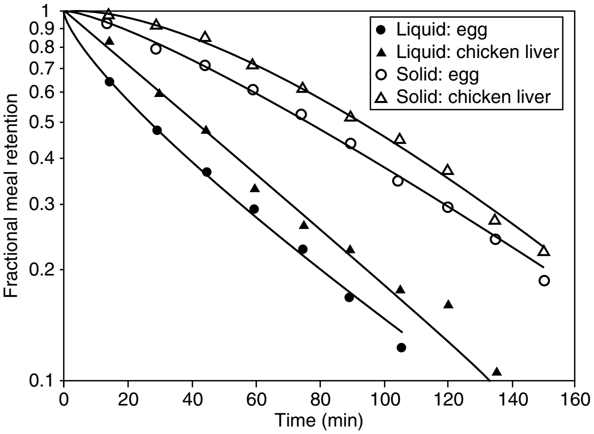
$$t_{lag} = \frac{\ln(b)}{k}$$

The above functions have been useful in describing the different patterns of emptying of the solid phase of gastric chyme (Siegel et al. 1988). The fractional retention of the solid phase exhibits a significant lag phase, which is described by the ratio of k to b and varies in duration according to the ease with which the solid phase undergoes trituration and digestion (Siegel et al. 1988). The lag phase is very small in the case of the liquid phase, and hence its fractional retention is described principally by a simple negative exponential function the slope of which approaches that of the latter part of the curve for the solid phase. This can be taken as indicating that, once it has been broken down to a suitable size, the solid phase will travel in concert with the liquid phase.

The function is useful for comparing the emptying of liquid and solid phase markers as it describes the loss of fixed amounts of liquid or solid phase labels and will include any effect of ongoing gastric secretion. However, the forms of the curves may be attenuated by the distance of the labelled material from the gamma counter, the anterior count rate tending to increase as labelled material moves from the fundus to the antrum (Siegel et al. 1988).

Given the curvilinear form of solid phase emptying, it is noteworthy that the half emptying time of the solid phase will not completely characterise the lag period or the actual rate of gastric emptying (Siegel et al. 1988).

Fig. 9.3 The mean gastric fractional meal retention values on a semilogarithmic plot for 24 subjects receiving either a Tc-99m containing egg sandwich ($n=14$) or Tc-99m in vivo-labelled chicken liver ($n=10$) and In-111 diethylenetriaminepentaacetate (DTPA) containing water. (Siegel et al. 1988)



The fact that the lag time varies with type of food (Siegel et al. 1988) (Fig. 9.3) and with particle size (Urbain et al. 1989; Weiner et al. 1981) suggests that gastric emptying of solid material may be governed in part by antral work. The fact that the emptying of liquid phase is linear fits in with the results of studies using a combination of high-resolution manometry and MRI (Indireshkumar et al. 2000), which show that antral contractile activity has little effect either on the gastric emptying of liquids or on the transit of solid matter that is of sufficient size to transit the pylorus. Hence as discussed earlier, the ‘pressure pump’ of fundic and corporal tone rather than the ‘peristaltic pump’ of antral phasic contraction provides the force to power the onflow of the liquid phase of chyme and of the contained fine particles into the duodenum (Indireshkumar et al. 2000).

9.3.5.2 Effect of Physical Properties of Contents on Gastric Outflow

A number of studies have examined the effects of the physical characteristics of gastric chyme on gastric emptying, particularly in regard to the possibility of manipulating food intake, subsequent fatty acid uptake, and glycaemic response (Dikeman and Fahey 2006). While the physical characteristics of gastric contents have been discussed in the first part of this book, it is nevertheless useful to revisit this topic insofar as a number findings that shed additional light on mechanisms underlying gastric processing and the onflow of the solid and liquid components of digesta from the stomach.

The assessment of the relative speed of onflow of solid and liquid phases from the human stomach is complicated by terminology and by methodological difficulties. With regard to the latter, it is important to consider the following problems when evaluating experimental work. Firstly, there may be elution of label from prelabelled particulate matter into the liquid phase (Houghton et al. 1989; Sagar et al. 1983). Secondly, a meal that has been ‘doped’ by adding a liquid label that binds to constituent particles may contain substantial residue of the label in the solid phase.

The use of the term fibre can relate to insoluble fibrous particles that form a suspension or mat, the components of which are capable of exerting direct force on mechanoreceptors. Alternatively it may be applied to soluble fibres (Dikeman and Fahey 2006), which dissolve in and augment the viscosity of the liquid phase causing it to offer generally increased resistance to the motion of suspended particulate matter, notably influencing the rate of settling of denser material into the greater curve of the stomach.

Onflow of Particulate and Liquid Phases

The consumption of viscous non-nutrient meals has been shown to delay gastric emptying, increase satiety and decrease hunger (Marciani et al. 2000, 2001b; Schwizer et al. 2002). The intensities of the latter sensations were correlated with a relative increase in functional antral diameter (Marciani et al. 2001b).

The emptying of digesta is decreased by the addition of soluble fibres, e.g. guar gum (Brown et al. 1988) or pectin (Di Lorenzo et al. 1988). It may be presumed that this results from a general effect of increasing the apparent viscosity of chyme. However, different types of fibre may generate different effects as is illustrated by a study of the gastric processing of three meals of equal apparent viscosity (Guerin et al. 2001). The passage of chyme from a meal that contained a high proportion of crude (insoluble) fibre, i.e. sugar beet pulp, was delayed relative to the passage of chyme from meals that contained either wheat bran or starch. Moreover, chyme from the pulp diet was more viscous than that from the other two meals (Guerin et al. 2001).

The overall excretion curves of the solid and liquid phases of gastric chyme were similar to those discussed earlier, as were those for the proximal stomach. However, the curves obtained from the distal portions of the stomach differed for the three meals (Guerin et al. 2001). The amount of the three meals remaining in the distal stomach was described by a biexponential function, presumably from a positively exponential rate of movement from the proximal to the distal stomach coupled with a negatively exponential exit from the distal stomach. It is notable that the peak in the proportion of the label in the distal stomach occurred later in the sugar beet diet and was lower than that for the wheat bran meal, but with a more tardy subsequent exponential decline. This was postulated by the authors to result from the sugar beet-derived chyme being subject to greater levels of retropulsion into the forestomach.

The well-documented ability of the stomach to selectively retain particles of a particular size has been as discussed earlier. This ability may be influenced by the viscosity of the liquid phase, presumably in slowing movement of particles during their retropulsion and during their subsequent gravity-assisted settling into the greater curvature. Both of these processes will influence the likelihood of a particle remaining suspended in the distal antrum in a position suitable for transiting the pylorus during a subsequent wave of distad propulsion. Hence, inert plastic particles of 3.5-mm diameter exit from the full stomachs of dogs when they are coadministered with guar gum but not when they are coadministered with saline

(Meyer et al. 1986). This occurs in spite of larger transpyloric flow rates obtained with saline.

References

Ahmed AB, Gilja OH, Gregersen H, Ødegaard S, Matre K (2006) In vitro strain measurement in the porcine antrum using ultrasound doppler strain rate imaging. Ultrasound Med Biol 32:513-522

Ahmed AB, Gilja OH, Hausken T, Gregersen H, Matre K (2009) Strain measurement during antral contractions by ultrasound strain rate imaging: Influence of erythromycin. Neurogastroenterol Mot 21:170-179

Andrews PL, Grundy D, Lawes IN (1980) The role of the vagus and splanchnic nerves in the regulation of intragastric pressure in the ferret. J Physiol 307:401-411

Ang D, Nicolai H, Vos R, Mimidis K, Akyuz F, Kindt S, Vanden Berghe P, Sifrim D, Depoortere I, Peeters T (2009) Influence of ghrelin on the gastric accommodation reflex and on meal-induced satiety in man. Neurogastroenterol Mot 21:528-533

Azpiroz F, Malagelada JR (1985) Physiological variations in canine gastric tone measured by an electronic barostat. Am J Physiol 248:G229-237

Balchum OJ, Weaver HM (1943) Pathways for pain from the stomach of the dog. Arch Neurol Psychiat 49:739-753

Bassotti G, Iantorno G, Fiorella S, Bustos-Fernandez L, Bilder CR (1999) Colonic motility in man: Features in normal subjects and in patients with chronic idiopathic constipation. Am J Gastroenterol 94:1760-1770

Becker JM, Kelly KA (1983) Antral control of canine gastric emptying of solids. Am J Physiol 245:G334-338

Bellisle F, Guy-Grand B, Le Magnen J (2000) Chewing and swallowing as indices of the stimulation to eat during meals in humans: Effects revealed by the edogram method and video recordings. Neurosci Biobehav Rev 24:223-228

Berstad A, Hausken T, Gilja OH, Thune N, Matre K, Ødegaard S (1994) Volume measurements of gastric antrum by 3-D ultrasonography and flow measurements through the pylorus by duplex technique. Dig Dis Sci 39:97-100

Berthoud HR, Hennig G, Campbell M, Volaufova J, Costa M (2002) Video-based spatio-temporal maps for analysis of gastric motility in vitro: Effects of vagal stimulation in guinea-pigs. Neurogastroenterol Mot 14:677-688

Björnhag G (1972) Separation and delay of contents in the rabbit colon. Swed J Agr Res 2:125–136

Björnhag G (1981) Separation and retrograde transport in the large intestine of herbivores. Livestock Prod Sci 8:351-360

Björnhag G (1987) Comparative aspects of digestion in the hindgut of mammals. The colonic separator mechanism (CSM)(a review). Dtsch Tierarztl Wochenschr 94:33-36

Björnhag G (1994) Adaptations in the large intestine allowing small animals to eat fibrous foods. In: Chivers DJ, Langer P (eds) The digestive system in mammals; food form and function. University Press, Cambridge pp 287-309

Blat S, Guerin S, Chauvin A, Bobillier E, Le Cloirec J, Bourguet P, Malbert CH (2001) Role of vagal innervation on intragastric distribution and emptying of liquid and semisolid meals in conscious pigs. Neurogastroenterol Mot 13:73-80

Brown BP, Schulze-Delrieu K, Schrier JE, Abu-Yousef MM (1993) The configuration of the human gastroduodenal junction in the separate emptying of liquids and solids. Gastroenterology 105:433-440

Brown NJ, Worlding J, Rumsey RDE, Read NW (1988) The effect of guar gum on the distribution of a radiolabelled meal in the gastrointestinal tract of the rat. Br J Nutr 59:223-231

Burton S, Washington N, Steele RJ, Musson R, Feely L (1995) Intragastric distribution of ion-exchange resins: A drug delivery system for the topical treatment of the gastric mucosa. J Pharm Pharmacol 47:901-906

Caldarella MP, Azpiroz F, Malagelada JR (2003) Antro-fundic dysfunctions in functional dyspepsia. Gastroenterology 124:1220-1229

Camilleri M, Malagelada JR, Brown ML, Becker G, Zinsmeister AR (1985) Relation between antral motility and gastric emptying of solids and liquids in humans. Am J Physiol 249:G580-585

Camilleri M, Prather CM (1994) Axial forces during gastric emptying in health and models of disease. Dig Dis Sci 39:14-17

Cannon WB (1898) The movements of the stomach studied by means of the Röntgen rays. Am J Physiol 1:359-382

Cannon WB (1902) The movements of the intestines studied by means of the Rontgen rays. Am J Physiol 6:251-277

Cannon WB (1912) Peristalsis, segmentation, and the myenteric reflex. Am J Physiol 30:114-128

Carleton M (1973) A survey of gross stomach morphology in New World Cricetinae (Rodentia, Muroidea), with comments on functional interpretations. Museum of Zoology, University of Michigan, Ann Arbor

Carlson HC, Code CF, Nelson RA (1966) Motor action of the canine gastroduodenal junction: A cineradiographic, pressure, and electric study. Dig Dis Sci 11:155-172

Castonguay TW, Kaiser LL, Stern JS (1986) Meal pattern analysis: Artifacts, assumptions and implications. Brain Res Bull 17:439-443

Cervero F (1991) Visceral pain: What's in the gut and what's in the brain. In: Taché Y, Wingate O (eds) Brain-gut interactions. CRC Press, Boca Raton, pp 339–346

Chey WY, Jin HO, Lee MH, Sun SW, Lee KY (2001) Colonic motility abnormality in patients with irritable bowel syndrome exhibiting abdominal pain and diarrhea. Am J Gastroenterol 96:1499-1506

Chivers DJ, Langer P (1994) The digestive system in mammals: Food, form, and function. Cambridge University Press, Cambridge

Collier G, Johnson DF, Mitchell C (1999) The relation between meal size and the time between meals: Effects of cage complexity and food cost. Physiol Behav 67:339-346

Collins PJ, Horowitz M, Chatterton BE (1988) Proximal, distal and total stomach emptying of a digestible solid meal in normal subjects. Br J Radiol 61:12-18

Collins PJ, Houghton LA, Read NW, Horowitz M, Chatterton BE, Heddle R, Dent J (1991) Role of the proximal and distal stomach in mixed solid and liquid meal emptying. Br Med J 32:615-619

Cork SJ, Hume ID, Faichney GJ (1999) Digestive strategies of nonruminant herbivores: The role of the hindgut. In: Jung H-JG, Fahey GC (eds) Nutritional ecology of herbivores. Am Soc Animal Sci, Savoy, pp 210-260

Cormack D, Ham A, Worth A (1987) Ham's histology. Lippincott Philadelphia

Coulie B, Camilleri M, Bharucha AE, Sandborn WJ, Burton D (2001) Colonic motility in chronic ulcerative proctosigmoiditis and the effects of nicotine on colonic motility in patients and healthy subjects. Aliment Pharmacol Ther 15:653-663

D'Antona G, Hennig GW, Costa M, Humphreys CM, Brookes SJH (2001) Analysis of motor patterns in the isolated guinea-pig large intestine by spatio-temporal maps. Neurogastroenterol Mot 13:483-492

de Castro JM (1988) Physiological, environmental, and subjective determinants of food intake in humans: A meal pattern analysis. Physiol Behav 44:651-659

De Schepper HU, Cremonini F, Chitkara D, Camilleri M (2004) Assessment of gastric accommodation: Overview and evaluation of current methods. Neurogastroenterol Mot 16:275-285

de Zwart IM, Haans JJL, Verbeek P, Eilers PHC, de Roos A, Masclee AAM (2007) Gastric accommodation and motility are influenced by the barostat device: Assessment with magnetic resonance imaging. Am J Physiol 292:G208-214

Di Lorenzo C, Williams C, Hajnal F, Valenzuela J (1988) Pectin delays gastric emptying and increases satiety in obese subjects. Gastroenterology 95:1211-1215

Dikeman CL, Fahey GC (2006) Viscosity as related to dietary fiber: A review. Crit Rev Food Sci Nutr 46:649-663

Dinning PG, Szczesniak MM, Cook IJ (2008) Twenty four hour spatiotemporal mapping of colonic propagating sequences provides pathophysiological insight into constipation. Neurogastroenterol Mot 20:1017-1021

Edelbroek M, Horowitz M, Maddox A, Bellen J (1992) Gastric emptying and intragastric distribution of oil in the presence of a liquid or a solid meal. J Nucl Med 33:1283-1290

Ehrlein HJ (1979) Caecum- und colonmotorik beim kaninchen. IWF, Göttingen, Germany, p 22:58

Ehrlein HJ, Reich H, Schwinger M (1982) Physiological significance of the contractions of the rabbit proximal colon. Q J Exp Psychol 67:407-417

Ehrlein HJ, Reich H, Schwinger M (1983) Colonic motility and transit of digesta during hard and soft faeces formation in rabbits. J Physiol 338:75-86

Ehrlein HJ, Ruoff G (1982) Cecal motility and flow of ingesta from the ileum to the cecum, appendix, and colon in rabbits. In: Wienbeck M (ed) Motility of the digestive tract. Raven New York, pp 475–481

Eisenberg RL (2002) Gastrointestinal radiology: A pattern approach. Lippincott Williams & Wilkins, Philadelphia

Faas H, Hebbard GS, Feinle C, Kunz P, Brasseur JG, Indireshkumar K, Dent J, Boesiger P, Thumshirn M, Fried M (2001) Pressure-geometry relationship in the antroduodenal region in humans. Am J Physiol 281:G1214-1220

Fioramonti J, Ruckebusch Y (1974a) La motricité caecale chez le lapin. I. Nature des contractions. Ann Rech Vétér 5:1-13

Fioramonti J, Ruckebusch Y (1974b) La motricité caecale chez le lapin. II. Variations d'origine alimentaire. Ann Rech Vétér 5:201-212

Fioramonti J, Ruckebusch Y (1976) La motricité caecale chez le lapin. III. Dualité de l'excrétion fécale. Ann Rech Vétér 7:281-295

Ford MJ, Camilleri M, Wiste JA, Hanson RB (1995) Differences in colonic tone and phasic response to a meal in the transverse and sigmoid human colon. Br Med J 37:264-269

Gregersen H (2003) Biomechanics of the gastrointestinal tract: New perspectives in motility research and diagnostics. Springer Verlag, New York

Gregersen H, Hausken T, Yang J, Odegaard S, Gilja OH (2006) Mechanosensory properties in the human gastric antrum evaluated using B-mode ultrasonography during volume-controlled antral distension. Am J Physiol 290:G876-882

Gregersen H, Kassab G (1996) Biomechanics of the gastrointestinal tract. Neurogastroenterol Mot 8:277-297

Groedel F (1924) Die roentgenuntersuchung des magens. Roentgendiagnostik in der inneren medizin. Lehmann Verlag, Munich, pp 502-505

Gruber P, Rubinstein A, Li VHK, Bass P, Robinson J (2006) Gastric emptying of nondigestible solids in the fasted dog. J Pharm Sci 76:117-122

Guerin S, Ramonet Y, LeCloarec J, Meunier-Salaun MC, Bourguet P, Malbert CH (2001) Changes in intragastric meal distribution are better predictors of gastric emptying rate in conscious pigs than are meal viscosity or dietary fibre concentration. Br J Nutr 85:343-350

Hausken T, Mundt M, Samsom M (2002) Low antroduodenal pressure gradients are responsible for gastric emptying of a low-caloric liquid meal in humans. Neurogastroenterol Mot 14:97-105

Hennig GW, Brookes SJH, Costa M (1997) Excitatory and inhibitory motor reflexes in the isolated guinea-pig stomach. J Physiol 501:197-212

Hennig GW, Costa M, Chen BN, Brookes SJH (1999) Quantitative analysis of peristalsis in the guinea-pig small intestine using spatio-temporal maps. J Physiol 517:575-590

Hertz AF, Newton A (1913) The normal movements of the colon in man. J Physiol 47:57-65

Hill AV (1970) First and last experiments in muscle mechanics. Cambridge University Press

Hinder RA, Kelly KA (1977) Canine gastric emptying of solids and liquids. Am J Physiol 233:G335-340

Hipper K, Ehrlein HJ (2001) Motility of the large intestine and flow of digesta in pigs. Res Vet Sci 71:93-100

Hoad CL, Rayment P, Spiller RC, Marciani L, Alonso BC, Traynor C, Mela DJ, Peters HPF, Gowland PA (2004) In vivo imaging of intragastric gelation and its effect on satiety in humans. J Nutr 134:2293-2300

Holzknecht G (1909) Die normale peristaltik des kolon. Munch Med Wochenschr 56:2401-2403

Hörnicke H, Björnhag G (1980) Coprophagy and related strategies for digesta utilization. In: Ruckebusch Y, Thivend P (eds) Digestive physiology and metabolism in ruminants. MTP Press, Lancaster, pp 707–730

Horowitz M, Jones K, Edelbroek MA, Smout AJ, Read NW (1993) The effect of posture on gastric emptying and intragastric distribution of oil and aqueous meal components and appetite. Gastroenterology 105:382-390

Houghton AD, Lazarus C, Liepins P, Allen S, Mason R, Clarke S (1989) Technetium-labelled bran to measure solid gastric emptying. Nucl Med Commun 10:486

Houghton LA, Mangnall YF, Read NW (1990) Effect of incorporating fat into a liquid test meal on the relation between intragastric distribution and gastric emptying in human volunteers. Gut 31:1226-1229

Hume ID (1999) Marsupial nutrition. Cambridge University Press, Cambridge

Indireshkumar K, Brasseur JG, Faas H, Hebbard GS, Kunz P, Dent J, Feinle C, Li M, Boesiger P, Fried M (2000) Relative contributions of ”pressure pump” and ”peristaltic pump” to gastric emptying. Am J Physiol 278:G604-616

Jacobshagen E (1937) Mittel- und enddarm. In: Bolk L, Göppert E, Kallius E, Lubosch W (eds) Handbuch der vergleichenden anatomie der wirbeltiere. Urban-Schwarzenberg, Berlin, pp 654–691

Janssen P, Lentle R, Hulls C, Ravindran V, Amerah A (2009) Spatiotemporal mapping of the motility of the isolated chicken caecum. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 179:593-604

Jefferson G (1915) The human stomach and the *canalis gastricus* (Lewis). J Anat Physiol 49:165-181

Kamba M, Seta Y, Kusai A, Ikeda M, Nishimura K (2000) A unique dosage form to evaluate the mechanical destructive force in the gastrointestinal tract. Int J Pharm 208:61-70

Kamba M, Seta Y, Kusai A, Nishimura K (2001) Evaluation of the mechanical destructive force in the stomach of dog. Int J Pharm 228:209-217

Kamba M, Seta Y, Kusai A, Nishimura K (2002) Comparison of the mechanical destructive force in the small intestine of dog and human. Int J Pharm 237:139-149

Karasov WH, Hume ID (1997) Vertebrate gastrointestinal system. In: Dantzler WH (ed) Handbook of physiology, section 13: Comparative physiology, vol 1. Oxford University Press, Oxford, pp 409-480

Karaus M, Sarna SK (1987) Giant migrating contractions during defecation in the dog colon. Gastroenterology 92:925-933

Keinke O, Ehrlein HJ (1983) Effect of oleic acid on canine gastroduodenal motility, pyloric diameter and gastric emptying. Q J Exp Physiol 68:675-686

Kelly KA (1980) Gastric emptying of liquids and solids: Roles of proximal and distal stomach. Am J Physiol 239:G71-76

Kiefer JC (2003) Molecular mechanisms of early gut organogenesis: A primer on development of the digestive tract. Dev Dyn 228:287-291

Kunz P, Feinle-Bisset C, Faas H, Boesiger P, Fried M, Steingoetter A, Schwizer W (2005) Effect of ingestion order of the fat component of a solid meal on intragastric fat distribution and gastric emptying assessed by MRI. J Magn Reson Imaging 21:383-390

Kwiatek MA, Steingoetter A, Pal A, Menne D, Brasseur JG, Hebbard GS, Boesiger P, Thumshirn M, Fried M, Schwizer W (2006) Quantification of distal antral contractile motility in healthy human stomach with magnetic resonance imaging. J Magn Reson Imaging 24:1101–1109

Lacy BE, Weiser K (2005) Gastric motility, gastroparesis, and gastric stimulation. Surg Clin North Am 85:967-987

Lammers W, Ver Donck L, Stephen B, Smets D, Schuurkes JAJ (2009) Origin and propagation of the slow wave in the canine stomach: The outlines of a gastric conduction system. Am J Physiol 296:G1200-1210

Lammers WJEP, Stephen B, Slack JR (2002) Similarities and differences in the propagation of slow waves and peristaltic waves. Am J Physiol 283:G778-786

Langer P (1988) The mammalian herbivore stomach: Comparative anatomy, function, and evolution. Gustav Fischer, Stuttgart

Langer P, Takács A (2004) Why are taeniae, haustra, and semilunar folds differentiated in the gastrointestinal tract of mammals, including man? J Morphol 259:308-315

Le Magnen J (1992) Neurobiology of feeding and nutrition. Academic, San Diego

Lefebvre RA, Dick JMC, Guerin S, Malbert CH (2005) Involvement of NO in gastric emptying of semi-solid meal in conscious pigs. Neurogastroenterol Mot 17:229-235

Lemann M, Flourie B, Picon L, Coffin B, Jian R, Rambaud J (1995) Motor activity recorded in the unprepared colon of healthy humans. Br Med J 37:649

Lentle RG, Hemar Y, Hall CE, Stafford KJ (2005) Periodic fluid extrusion and models of digesta mixing in the intestine of a herbivore, the common brushtail possum (*trichosurus vulpecula*). J Comp Physiol B175:337-347

Lentle RG, Janssen PWM (2008) Physical characteristics of digesta and their influence on flow and mixing in the mammalian intestine: A review. J Comp Physiol B178:673-690

Lentle RG, Janssen PWM, Asvarujanon P, Chambers P, Stafford KJ, Hemar Y (2007) High definition mapping of circular and longitudinal motility in the terminal ileum of the brushtail possum *trichosurus vulpecula* with watery and viscous perfusates. J Comp Physiol B177:543-556

Lentle RG, Janssen PWM, Asvarujanon P, Chambers P, Stafford KJ, Hemar Y (2008) High definition spatiotemporal mapping of contractile activity in the isolated proximal colon of the rabbit. J Comp Physiol B178:257-268

Lentle RG, Janssen PWM, Goh K, Chambers P, Hulls C (2010) Quantification of the effects of the volume and viscosity of gastric contents on antral and fundic activity in the rat stomach maintained *ex vivo*. Dig Dis Sci DOI 10.1007/s10620-010-1164-y:1-12

Lind JF, Duthie HL, Schlegel JF, Code CF (1961) Motility of the gastric fundus. Am J Physiol 201:197-202

Lu Y, Owyang C (1995) Secretin at physiological doses inhibits gastric motility via a vagal afferent pathway. Am J Physiol 268:G1012-1016

Mackenna BR, McKirdy HC (1972) Peristalsis in the rabbit distal colon. J Physiol 220:33-54

Maes BD, Hiele MI, Geypens BJ, Ghoos YF, Rutgeerts PJ (1998) Gastric emptying of the liquid, solid and oil phase of a meal in normal volunteers and patients with Billroth II gastrojejunostomy. Eur J Clin Invest 28:197-204

Malbert CH, Mathis C (1994) Antropyloric modulation of transpyloric flow of liquids in pigs. Gastroenterology 107:37-46

Malbert CH, Ruckebusch Y (1991) Relationships between pressure and flow across the gastroduodenal junction in dogs. Am J Physiol 260:G653-657

Mandrek K, Golenhofen K (1990) Phasic-rhythmical and tonic components in gastrointestinal motility. Prog Clin Biol Res 327:463-481

Marciani L, Gowland PA, Fillery-Travis A, Manoj P, Wright J, Smith A, Young P, Moore R, Spiller RC (2001a) Assessment of antral grinding of a model solid meal with echo-planar imaging. Am J Physiol 280:G844-849

Marciani L, Gowland PA, Spiller RC, Manoj P, Moore RJ, Young P, Al-Sahab S, Bush D, Wright J, Fillery-Travis AJ (2000) Gastric response to increased meal viscosity assessed by echo-planar magnetic resonance imaging in humans. J Nutr 130:122-127

Marciani L, Gowland PA, Spiller RC, Manoj P, Moore RJ, Young P, Fillery-Travis AJ (2001b) Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. Am J Physiol 280:G1227-1233

Marciani L, Young P, Wright J, Moore R, Coleman N, Gowland PA, Spiller RC (2001c) Antral motility measurements by magnetic resonance imaging. Neurogastroenterol Mot 13:511-518

McIntyre A, Vincent RM, Perkins AC, Spiller RC (1997) Effect of bran, ispaghula, and inert plastic particles on gastric emptying and small bowel transit in humans: The role of physical factors. Br Med J 40:223-227

McTigue DM, Rogers RC (1995) Pancreatic polypeptide stimulates gastric motility through a vagal-dependent mechanism in rats. Neurosci Lett 188:93-96

Meyer JH (1991) The physiology of gastric motility and gastric emptying. In: Yamada T (ed) Textbook of gastroenterology. JB Lippincott, Philadelphia, pp 137–157

Meyer JH, Gu Y, Elashoff J, Reedy T, Dressman J, Amidon G (1986) Effects of viscosity and fluid outflow on postcibal gastric emptying of solids. Am J Physiol 250:G161-164

Meyer JH, Thomson JB, Cohen MB, Shadchehr A, Mandiola SA (1979) Sieving of solid food by the canine stomach and sieving after gastric surgery. Gastroenterology 76:804-813

Meyers MA, Volberg F, Katzen B, Abbott G (1973) Haustral anatomy and pathology: A new look. II. Roentgen interpretation of pathological alterations. Radiology 108:505-512

Miller J, Kauffman G, Elashoff J, Ohashi H, Carter D, Meyer JH (1981) Search for resistances controlling canine gastric emptying of liquid meals. Am J Physiol 241:G403-415

Mimura T, Emanuel A, Kamm MA (2002) Pathophysiology of diverticular disease. Best Pract Res Clin Gastroenterol 16:563-576

Mittal RK, Liu J, Puckett JL, Bhalla V, Bhargava V, Tipnis N, Kassab G (2005) Sensory and motor function of the esophagus: Lessons from ultrasound imaging. Gastroenterology 128:487-497

Morgan KG, Szurszewski JH (1980) Mechanisms of phasic and tonic actions of pentagastrin on canine gastric smooth muscle. J Physiol 301:229-242

Morrison JFB (1977) The afferent innervation of the gastrointestinal tract. In: Brooks FP, Evers PW (eds) Nerves and the gut. Slack, Thorofare, NJ pp 297-326

Mundt MW, Hausken T, Samsom M (2002) Effect of intragastric barostat bag on proximal and distal gastric accommodation in response to liquid meal. Am J Physiol 283:G681-686

Nguyen NQ, Fraser RJ, Bryant LK, Holloway RH (2007) Functional association between proximal and distal gastric motility during fasting and duodenal nutrient stimulation in humans. Neurogastroenterol Mot 19:638-645

Novitol R, Coffin B, Azpiroz F, Mearin F, Serra J, Malagelada JR (1995) Gastric tone determines the sensitivity of the stomach to distension. Gastroenterology 108:330-336

Pal A, Brasseur JG, Abrahamsson B (2007) A stomach road or "magenstrasse" for gastric emptying. J Biomech 40:1202-1210

Pal A, Indireshkumar K, Schwizer W, Abrahamsson B, Fried M, Brasseur JG (2004) Gastric flow and mixing studied using computer simulation. Proc R Soc Lond B Biol Sci 271:2587-2594

Paterson CA, Anvari M, Tougas G, Huizinga JD (2000) Determinants of occurrence and volume of transpyloric flow during gastric emptying of liquids in dogs. Dig Dis Sci 45:1509-1516

Penry DL, Jumars PA (1986) Chemical reactor analysis and optimal digestion. Bioscience 36:310-315

Pera P, Bucca C, Borro P, Bernocco C, De Lillo A, Carossa S (2002) Influence of mastication on gastric emptying. J Dent Res 81:179-181

Pescatori M, Marsicano B, Mancinelli R, Salinari S, Bertuzzi A (1980) Control of peristalsis in the isolated rabbit colon. In: Christensen J (ed) Gastrointestinal motility. Raven Press, New York, pp 479-486

Piessevaux H, Tack J, Wilmer A, Coulie B, Geubel A, Janssens J (2001) Perception of changes in wall tension of the proximal stomach in humans. Gut 49:203-208

Pluja L, Alberti E, Fernandez E, Mikkelsen HB, Thuneberg L, Jimenez M (2001) Evidence supporting presence of two pacemakers in rat colon. Am J Physiol 281:G255-266

Powley TL, Phillips RJ (2004) Gastric satiation is volumetric, intestinal satiation is nutritive. Physiol Behav 82:69-74

Prove J, Ehrlein HJ (1982) Motor function of gastric antrum and pylorus for evacuation of low and high viscosity meals in dogs. Gut 23:150-156

Ramkumar D, Schulze KS (2005) The pylorus. Neurogastroenterol Mot 17:22-30

Rao SSC, Sadeghi P, Beaty J, Kavlock R, Ackerson K (2001) Ambulatory 24-h colonic manometry in healthy humans. Am J Physiol 280:G629-639

Rao SSC, Vemuri S, Harris B, Schulze K (2002) Fundic balloon distension stimulates antral and duodenal motility in man. Dig Dis Sci 47:1015-1019

Ritchie JA (1968a) Colonic motor activity and bowel function. I. Normal movement of contents. Gut 9:442-456

Ritchie JA (1968b) Colonic motor activity and bowel function. II. Distribution and incidence of motor activity at rest and after food and carbachol. Gut 9:502-511

Ritchie JA, Truelove SC, Ardran GM, Tuckey MS (1971) Propulsion and retropulsion of normal colonic contents. Dig Dis Sci 16:697-704

Ropert A, des Varannes SB, Bizais Y, Rozé C, Galmiche JP (1993) Simultaneous assessment of liquid emptying and proximal gastric tone in humans. Gastroenterology 105:667-674

Ross M, Pawlina W (2006) Histology: A text and atlas: With correlated cell and molecular biology. Lippincott Williams & Wilkins USA

Ross MW, Cullen KK, Rutkowski JA (1990) Myoelectric activity of the ileum, cecum, and right ventral colon in ponies during interdigestive, nonfeeding, and digestive periods. Am J Vet Res 51:561-566

Sagar S, Grime JS, Little W, Patten M, Gulliford P, Critchley M, Bennett R, Shields R (1983) Technetium-99m labelled bran: A new agent for measuring gastric emptying. Clin Radiol 34:275-278

Sakaguchi E (2003) Digestive strategies of small hindgut fermenters. Anim Sci J 74:327-337

Sakaguchi T, Aono T, Ohtake M, Sandoh N (1994) Interaction of glucose signals between the nucleus of the vagus nerve and the portal vein area in the regulation of gastric motility in rats. Brain Res Bull 33:469-471

Sarna S, Latimer P, Campbell D, Waterfall WE (1982) Electrical and contractile activities of the human rectosigmoid. Br Med J 23:698-705

Sarna SK (1993) Gastrointestinal longitudinal muscle contractions. Am J Physiol 265:G156-164

Sarna SK, Shi XZ (2006) Function and regulation of colonic contractions in health and disease. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Academic Press, San Diego, pp 965–993

Sasaki Y, Hada R, Nakajima H, Munakata A (1996) Difficulty in estimating localized bowel contraction by colonic manometry: A simultaneous recording of intraluminal pressure and luminal calibre. Neurogastroenterol Mot 8:247-253

Savoye-Collet C, Savoye G, Smout A (2003) Determinants of transpyloric fluid transport: A study using combined real-time ultrasound, manometry, and impedance recording. Am J Physiol 285:G1147-1152

Savoye G, Savoye-Collet C, Oors J, Smout A (2003) Interdigestive transpyloric fluid transport assessed by intraluminal impedance recording. Am J Physiol 284:G663-669

Schirra J, Houck P, Wank U, Arnold R, Goke B, Katschinski M (2000) Effects of glucagon-like peptide-1 (7-36) amide on antro-pyloro-duodenal motility in the interdigestive state and with duodenal lipid perfusion in humans. Gut 46:622-631

Schulze-Delrieu K (1999) Visual parameters define the phase and the load of contractions in isolated guinea pig ileum. Am J Physiol 276:G1417-1424

Schulze-Delrieu K, Brown BP, Lange W, Custer-Hagen T, Lu C, Shirazi S, Lepsien G (1996a) Volume shifts, unfolding and rolling of haustra in the isolated guinea pig caecum. Neurogastroenterol Mot 8:217-225

Schulze-Delrieu K, Cook J, Herman B, Shirazi S, Brown B (1996b) Contractions in the isolated cat stomach produce retrograde jets which disperse digestible particles. Gastroenterology 110:A754

Schulze-Delrieu K, Herman RJ (1998) Changes in the position and angulation of incisura during contractions of the isolated cat stomach. Neurogastroenterol Mot 10:281-288

Schulze-Delrieu K, Herman RJ, Shirazi SS, Brown BP (1998) Contractions move contents by changing the configuration of the isolated cat stomach. Am J Physiol 274:G359-369

Schulze K (2006) Imaging and modelling of digestion in the stomach and the duodenum. Neurogastroenterol Mot 18:172-183

Schwarz G (1911) Zur physiologie und pathologie der menschlichen dickdarmbewegungen. Munch Med Wochenschr 68:1489-1494

Schwizer W, Fraser R, Borovicka J, Asal K, Crelier G, Kunz P, Boesiger P, Fried M (1996) Measurement of proximal and distal gastric motility with magnetic resonance imaging. Am J Physiol 271:G217-222

Schwizer W, Steingötter A, Fox M, Zur T, Thumshirn M, Bösiger P, Fried M (2002) Non-invasive measurement of gastric accommodation in humans. Gut 51:i59-i62

Siegel JA (1985) The effect of source size on the buildup factor calculation of absolute volume. J Nucl Med 26:1319-1322

Siegel JA, Urbain JL, Adler LP, Charkes ND, Maurer AH, Krevsky B, Knight LC, Fisher RS, Malmud LS (1988) Biphasic nature of gastric emptying. Br Med J 29:85-89

Siegel JA, Wu RK, Maurer AH (1985) The buildup factor: Effect of scatter on absolute volume determination. J Nucl Med 26:390-394

Smith GP (1998) Satiation: From gut to brain. Oxford University, New York

Smith TK, Reed JB, Sanders KM (1987) Interaction of two electrical pacemakers in muscularis of canine proximal colon. Am J Physiol 252:C290-299

Sperber I, Björnhag G, Ridderstråle Y (1983) Function of proximal colon in lemming and rat. Swed J Agr Res 13:243-256

Stevens CE, Hume ID (1995) Comparative physiology of the vertebrate digestive system. Cambridge University Press, New York

Takahashi T, Owyang C (1997) Characterization of vagal pathways mediating gastric accommodation reflex in rats. J Physiol 504:479-488

Takahashi T, Owyang C (1999) Mechanism of cholecystokinin-induced relaxation of the rat stomach. J Auton Nerv Syst 75:123-130

Talley NJ (2003) Diabetic gastropathy and prokinetics. Am J Gastroenterol 98:264-271

Toyama T, Blickman JR (1971) Serial roentgenographic observations on the passage of foods through the human stomach. Radiol Clin Biol 40:29-38

Tuleu C, Andrieux C, Boy P, Chaumeil JC (1999) Gastrointestinal transit of pellets in rats: Effect of size and density. Int J Pharm 180:123-131

Urbain JLC, Siegel JA, Charkes ND, Maurer AH, Malmud LS, Fisher RS (1989) The two-component stomach: Effects of meal particle size on fundal and antral emptying. Eur J Nucl Med 15:254-259

Van den Elzen BDJ, Bennink RJ, Wieringa RE, Tytgat GNJ, Boeckxstaens GEE (2003) Fundic accommodation assessed by spect scanning: Comparison with the gastric barostat. Gut 52:1548-1554

Vassallo MJ, Camilleri M, Prather CM, Hanson RB, Thomforde GM (1992) Measurement of axial forces during emptying from the human stomach. Am J Physiol 263:G230-239

Wang XY, Lammers W, Bercik P, Huizinga JD (2005) Lack of pyloric interstitial cells of Cajal explains distinct peristaltic motor patterns in stomach and small intestine. Am J Physiol 289:G539-549

Weiner K, Graham L, Reedy T, Elashoff J, Meyer JH (1981) Simultaneous gastric emptying of two solid foods. Gastroenterology 81:257-266

Weissberg H (1937) Beitrag zur morphologie des menschlichen dickdarms. Anat Embryol (Berl) 107:738-781

Wilbur BG, Kelly KA (1973) Effect of proximal gastric, complete gastric, and truncal vagotomy on canine gastric electric activity, motility, and emptying. Ann Surg 178:295-303

Chapter 10
Flow, Mixing and Absorption at the Mucosa

Contents

10.1 Introduction

Ultimately, it is the gastrointestinal mucosa that absorbs the nutrients that are liberated from food by the process of digestion. The function of the mucosa is complicated by the presence of a protective layer of mucin, which is interposed between the lumen and the apices of enterocytes, and by the variations in the sizes of the intercellular spaces between the component cells of the mucosa.

R. G. Lentle, P. W. M. Janssen, *The Physical Processes of Digestion,*
DOI 10.1007/978-1-4419-9449-3_10, © Springer Science+Business Media, LLC 2011

The molecular structure of the mucin layer (Cone 2009), along with the differing biochemical characteristics of enzymes that are secreted on either side of it, lead to the formation of a subsidiary compartment adjacent to the intestinal mucosa (Pappenheimer 2001b). Macromolecular nutrient substrates and the enzymes that digest them are constrained to the lumen by the mucin layer, while the soluble oligomeric products of lumen digestion are able to enter the submucous space and undergo further enzymatic breakdown into constituent monomers. The enzymes that conduct the latter are bound to vesicles, which are shed from apical microvilli (McConnell et al. 2009) and may be too large to transit the mucin layer and enter the lumen. Broadly speaking, these dynamics result in the sequential digestion of nutrients from macromolecules to oligomers and from oligomers to monomers in subsidiary compartments, a strategy that seems to occur widely in animals although rarely incorporated into models. For instance, a form of compartmentation also occurs in insects in which a chitinous peritrophic membrane is secreted around incoming food boluses and allows the ingress of enzymes and the egress of oligomeric products, which are subsequently digested in the unrestricted fluid phase at the mucosal interface (Terra 2001).

Variation in the sizes of tight junctions between the component cells of the mucosa adds a further dimension of complexity in allowing interdiffusion of the contents of the basolateral interspaces with those of the apicolateral interspaces between adjacent enterocytes. The dynamic regulation of this interconnection can, on occasion, allow the transit of water and contained solutes between the two spaces, the direction of movement depending on the osmotic difference between the two. Hence the absorption of nutrients is not entirely dictated by the presence of active and/or passive transporter systems in the apical membranes of enterocytes and may on occasion be predominantly via the paracellular spaces. The complex dynamics that result from the operation of these two processes are imperfectly understood.

In this chapter, we shall first explore what is known of these dynamics in regard to physiological outcomes, such as the presence of an ‘unstirred water layer’, (UWL) and subsequently examine the features that may influence mixing and absorption. This examination will include the features of the mucin layer and the enteral microflora that live upon it.

10.2 Permeability and the Unstirred Water Layer

The rate at which soluble substances undergo mass transfer from the lumen across a more or less stagnant layer of water and mucous, and are passively absorbed at the apical membrane can be measured as the effective intestinal permeability (P_{eff}). If a segment of intestine with radius R and length L is perfused with a solution at a volumetric flow rate Q, then at steady state, the permeability is given by (Amidon et al. 1980):

$$P_{eff} = \frac{Q(C_{in} - C_{out})/C_{out}}{2\pi RL}$$

where C_{in} and C_{out} are solute concentrations at the inlet and outlet. This expression assumes the surface area available for absorption is that of a cylinder, i.e. ignores any effect of villi on surface area. The total diffusional resistance ($1/P_{eff}$) is the sum of the diffusional resistance from the lumen to the compartment beneath the mucus layer ($1/P_{aq}$) and the resistance across the apical membrane of the enterocyte ($1/P_m$) (Johnson and Amidon 1988):

$$\frac{1}{P_{eff}} = \frac{1}{P_{aq}} + \frac{1}{P_m}$$

If a solute is able to rapidly permeate through the apical membrane of the component enterocytes ($P_{eff} \geq 2 \times 10^{-4}$ cm/s) (Fagerholm and Lennernäs 1995) then the overall permeability of the system will approximate to P_{aq} (Winne 1978):

$$P_{eff} \approx P_{aq}$$

The resistance to mass transfer from the lumen to the submucus compartment is often expressed as the equivalent thickness *(δ)* of a UWL. This can be calculated using the known rate of diffusion of the solute in water *(D)*:

$$\delta = \frac{D}{P_{aq}} \approx \frac{D}{P_{eff}}$$

A number of physical components contribute to the UWL and need to be considered when interpreting measurements of δ. The estimated operational thickness of the small intestinal UWL varies between 74–600 µm according to the technique by which it is measured, the rate of perfusion of the solution (Debnam and Levin 1975; Fagerholm and Lennernäs 1995; Read et al. 1976; Westergaard and Dietschy 1974; Winne 1978; Winne et al. 1979) and the solute used (Pohl et al. 1998). Specifically, measurements of δ do not consistently reflect the thickness of the mucin layer that overlies the apices of enterocytes.

Some of these components contributing to the UWL may not be well defined. These include the effects of structures such as villi (Ryu and Grim 1982) and their constituent layers, the microvilli, glycocalyx and adherent mucus (DeSimone 1983; Smithson et al. 1981; Westergaard et al. 1986). The measurement of δ may be influenced by the rate at which water circulates within the intervillous space, and by the magnitude and the direction of the net transepithelial flow of water (Gruzdkov et al. 1989), along with any consequent effect on the diffusion gradients of contained solutes (Pappenheimer 2001b) as result of solute drag (Mullen et al. 1985).

While turbulent flow through a tube is very effective at mixing the contents, a poorly mixed boundary layer will persist along its wall. The thickness of this boundary layer is reduced by increasing the flow rate of the contents (Karlsson and Artursson 1991) but it is important to note that the layer of mucus will not behave in the same fashion as a boundary layer, i.e. it will not be reduced in this manner. In addition, the measured thickness of the UWL will increase when the development of turbulence is impeded by high viscosity of the luminal contents and/or low levels of gut motility (Levitt et al. 1987).

10.2.1 Effect of Villi

The presence of villi has long been thought to increase the effective surface area of the intestinal mucosa that is available for absorption of nutrients. However, the lateral surfaces of villi lie at right angles to the direction of flow of digesta through the intestinal lumen and to the direction of propagation of contractile activity. Hence the distance between adjacent villi (the intervillous space) may vary in size with tonic or phasic contraction of the intestinal walls, and the liquid phase of digesta in the intervillous space may not be subject to the same level of mixing as are the contents of the lumen (Levitt et al. 1987). Consequently, the relative stasis between adjacent villi may contribute to the UWL.

Such considerations have led a number of workers to the opinion that the absorption of highly permeable solutes occurs principally at the tips of villi (Chang and Rao 1994; Strocchi and Levitt 1993) with the bulk of nutrient solutes being absorbed within the terminal 5% of the length of the villus. In this case, less than 2% of cells in the intervillous spaces would contribute to the absorption of common actively absorbed nutrients, such as glucose. Such a situation would validate the representation of the absorptive surface area of a segment of small intestine as a simple cylinder, as has been hypothesised (Amidon et al. 1980). This hypothesis is supported by a body of work showing that for the jejunum of conscious human subjects, the values of δ that are calculated using the surface area of a simple cylinder approach the physical thickness of the mucus gel layer found in biopsies from the human jejunum (Fagerholm and Lennernäs 1995; Sarosiek et al. 1991). However, the steady-state assumption on which this method is based may be incorrect. The stasis of the liquid in the intervillous spaces may be disturbed during peristalsis as a result of concomitant changes in the volume of intervillous spaces owing to changes in the diameter or length of the intestinal wall.

Early workers carrying out experiments on permeability reported that increases in the rate at which the small intestine was perfused caused the intestine to become distended, the inner cylindrical surface area to increase (Holzheimer and Winne 1989), and the overall rate of absorption to increase (Levitt et al. 1987), but the effective mucosal permeability was reduced (Levitt et al. 1987; Winne 1979). On the basis of these findings, it was hypothesised that the increase in intestinal radius and cylindrical surface area that occurred when the intestine was distended would reduce permeability by creating a larger pool of unstirred liquid between adjacent villi, which would increase the effective thickness of the UWL and overall diffusion resistance (Winne 1989). Incumbent in this hypothesis was the assumption that little or no convective mixing occurred between adjacent villi (Ryu and Grim 1982).

The validity of the latter findings was later challenged on the basis that they were obtained from anaesthetised animals (Fagerholm and Lennernäs 1995). Moreover, these workers reported that in similar experiments on permeability, the effective thickness of the UWL decreased when perfusion was increased, presumably by increasing the effective area of the intervillous space (Fagerholm and Lennernäs 1995; Harris and Kennedy 1988). Such an increase could be caused by increasing the level of mixing occurring within intervillous spaces.

Further support for the hypothesis that permeability increases with distension comes from work investigating the effect of flow rate on the permeability of jejunal mucosa to a series of solutes of differing molecular size in conscious human subjects. This work showed that small intestinal permeability to solutes of lower molecular size increased with distension (Fine et al. 1995). These findings led to the conclusion that mean pore size decreased as a result of increased separation of villi and increased perfusion of the intervillous space reducing the mean length of the diffusion path to, and allowing greater levels of diffusion of smaller molecules through, smaller pores on the lateral surfaces of villi (Fine et al. 1995) (see also below).

10.2.2 Effect of Solute Diffusion Coefficient and Solvent Drag

As noted earlier, the thickness of the UWL depends upon the diffusion coefficient, which differs between molecular species (Pohl et al. 1998). Hence determinations of thickness of the UWL show that very small molecules with high diffusion coefficients, such as carbon monoxide ($D = 2.7 \times 10^{-5}$ cm^2/s), seem to encounter an apparently thicker UWL than do larger molecules with lower diffusion coefficients such as warfarin ($D = 7.2 \times 10^{-6}$ cm^2/s). This effect may result from differences in the effective distance through which a particle must travel to reach the apical membrane as result of solvent drag, a movement induced in solute molecules by the osmotic transit of volumes of the containing solvent (water) through the apical membranes of enterocytes (Pappenheimer 2001b). Hence, as a consequence of their lower diffusion rate, larger particles are influenced to a greater extent by solvent drag and are drawn towards the apical membrane more effectively than are smaller more mobile particles, which in effect traverse longer distances (Pohl et al. 1998).

The ongoing movement of slowly or non-absorbed solutes with solvent (solvent drag) (Schafer and Andreoli 1986) across the UWL will cause these solutes to accumulate at the enterocyte apex altering the concentration gradient across the UWL (Fettiplace and Haydon 1980). Thus the rate of diffusion of the solute from the lumen to the membrane will be reduced as the concentration at the membrane (C_s) increases. This effect can be stated in terms of the variation of solute concentration $C(x)$ with distance x from the membrane (Pohl et al. 1998):

$$C(x) = C_s \, e^{\left(\frac{-vx}{D} + \frac{ax^3}{3D}\right)}$$

where D is the diffusion coefficient, v the linear velocity of the osmotic volume flow and a is the velocity of a stirring parameter.

10.2.3 Effect of Local Increase in Nutrient Concentration at the Brush Border

In the section above, it was noted that solvent drag may cause the concentration of a non-membrane-penetrating solute to rise in the UWL in the immediate vicinity of the membrane and alter the concentration gradient across the UWL (Fettiplace and Haydon 1980). While such a change may act to reduce the diffusion gradient of a substance across the UWL, it will augment the gradient across the apical membranes of the enterocytes in favour of cellular absorption, provided that it is present only in low concentrations in the cytosol (Pappenheimer 2001b).

The fact that locally acting enzymes from the brush border (McConnell et al. 2009) promote digestion of nutrients from oligomer to monomer at or adjacent to the brush border (see the later section on enteral microvilli) may further augment the efficiency of such absorption (Pappenheimer 2001b), in effect decreasing the effective thickness of the UWL by locally increasing the concentration gradient across the apical membrane. Hence the hydrolysis of nutrient oligomers, such as maltose, above the brush border will create relatively high local concentrations of monomers and augment the diffusion gradient across the apical membrane of the enterocyte. The fact that levels of the 'parent' oligomer are able to be maintained at this site by diffusion from the lumen through the UWL depends on the relationship between molecular size, the rate of diffusion and the rate of generation of monomers by enzymatic action. Thus diffusion rate is proportional to $(MW)^{0.5}$ while the number of monomers that is released from an oligomer is directly proportional to its molecular weight, i.e. $(MW)^1$ (Pappenheimer 2001b).

10.2.4 Effect of Enterocyte Membrane Permeability

The discussion above indicates that solvent drag causes the permeability of a substance though the apex of the enterocyte to influence the diffusion gradient for that substance across the UWL, i.e. between the lumen and the apical membrane of the enterocyte. Hence, in the situations where monomers that have transited the UWL are rapidly actively transported across the apical membrane, unfavourable lumen to brush border concentration gradients, which result from solvent drag, will not build up, provided the rate of membranous absorption is greater than the rate at which the monomers arrive or are generated at the membrane surface (Fig. 10.1a). In cases where the rates of membrane transport and permeability of the membrane to a solute are lower, the solute may accumulate at those sites in the villous architecture where the UWL is thinnest and the rate of arrival of monomers at the membrane is highest, i.e. at the villous tips. In cases where membrane permeability is lower still, the solute may start to accumulate in areas where the UWL is thicker, i.e. in the apical and adjacent intervillous UWLs, thus extending the area in which a transmembrane gradient is established to facilitate diffusion and absorption (Fig. 10.1b). In the extreme

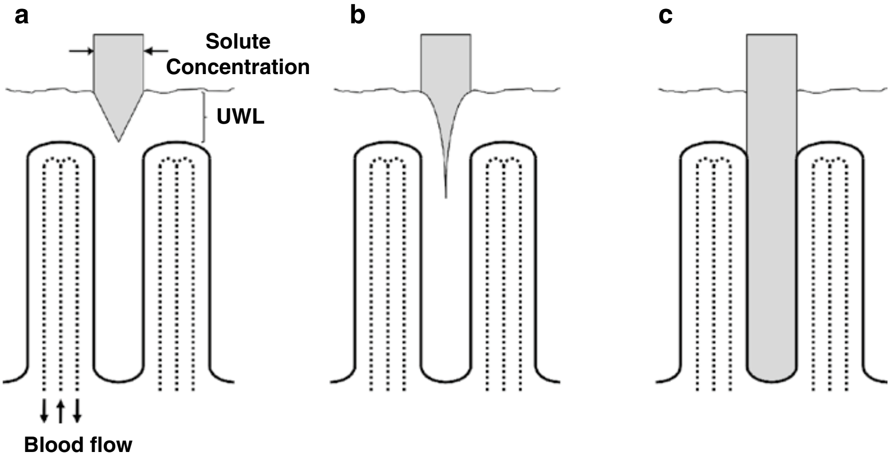


Fig. 10.1 Showing different models for the aqueous diffusion barrier for glucose. The concentration of solute is represented by the width of the *shaded* region. **a** Rapid epithelial transport results in a negligible solute concentration at the villous tips and the uptake rate is only limited by the unstirred layer over the villous tips. **b** Epithelial transport somewhat limits absorption, solute concentration at the tips rises and solute diffuses into intervillous space where additional absorption occurs. **c** This is the extreme case in which the membrane permeability is so low that the solute concentration is constant throughout the unstirred water layer. (Levitt et al. 1990)

case of a solute that is very poorly absorbed, the UWL across the entire intervillous area will become saturated, and the area from which absorption can occur will be correspondingly increased (Levitt et al. 1990).

10.2.5 Effect of Molecular Size and Interaction with Solvent Drag

The rates of diffusion of nutrient molecules through passive pores in the apical membranes of enterocytes are inversely related to the square root of their molecular weight, provided their molecular weight is less than around 15 times the molecular weight of the solvent in which they are dissolved (Stein 1967). Similarly, they are inversely related to the cube root of the molecular size for nutrient molecules greater than 15 times the size of that of the solvent. These relationships can be used to predict the ratio of the rates of diffusive flux for two molecular probes that are commonly used to explore intestinal permeability, i.e. mannitol and lactulose. This calculation gives a ratio value of between 0.73 and 0.81. A similar calculation based on the Stokes–Einstein diffusion relationship, i.e. the relationship between the molecular radius and diffusion, similarly predicts a lactose to mannitol diffusion ratio of 0.71–0.80 (Menzies 1984). However, the ratio of the flux rates of these molecular probes across the small intestinal mucosa of healthy human subjects, estimated from urinary excretion, is much lower, i.e. around 0.025 (Bijlsma et al. 1995). Given that the rates of lumen digestion, metabolism and renal handling of the two compounds do not differ (Travis and Menzies 1992), the difference from theoretical values based on molecular diffusion suggests that there may be a range of different pore sizes in small intestinal mucosa, and that there are large numbers of small pores that admit mannitol but exclude lactulose, but smaller numbers of larger pores that admit both lactulose and mannitol. It is generally accepted that the larger pores comprise the intercellular tight junctions but there are conflicting views regarding the location of the smaller pores and two theories have been advanced. The transcellular theory suggests that ‘permeation of water-soluble, lipid-insoluble molecules of mannitol-like radius or below can take place freely through numerous small ‘water pores’ situated in the cell membrane of the mucosal enterocytes, whereas those of greater size can only pass very slowly through large paracellular water channels of low incidence (Menzies 1984). The paracellular theory suggests that these differences arise solely from differences in the morphology of tight junctions between the component enterocytes cells of the villus and those of the associated crypts of Lieberkühn. The former are postulated to be smaller, and therefore, permeable to mannitol and smaller-sized molecules, while the latter are larger and permeable to both lactulose and mannitol, but are less accessible (Hollander 1992) as they have a thicker overlying UWL.

Studies of the rates of flux of mannitol and lactulose in sheets of stripped intestinal mucosa from various animal species mounted in an Ussing chamber have shown that mucosal flux ratios approach those predicted from diffusion ratios, but in humans, the flux rates differ from those conducted in vitro, chiefly on account of the lower flux rate of mannitol in vivo (Bijlsma et al. 1995). This finding prompted the authors to speculate that mannitol (but not lactulose) absorption was compromised in isolated mucosa as a result of the impairment of solvent drag. Further they suggested that high levels of solvent drag in vivo in human subjects resulted from local hyperosmolality of the cells in the villous tips due to the operation of a countercurrent multiplication system based on the orientation of flow in villous capillaries and arterioles (see below).

10.3 Mucosal Morphology

Although the gross morphology of the mucosa has been well described, it has long been recognised that the exigencies of histological and other types of sample preparation may induce a degree of artefact. Recent advances have made it possible to study the micro-morphology of the mucosa in vivo in human and animal subjects. The advent of magnifying upper endoscopy (Cammarota et al. 2004) and confocal endomicroscopy (Odagi et al. 2007), along with the use of in vivo staining techniques, may soon allow mucosal morphology to be described in greater detail, notably the dimensions of the intervillous space and the level of convective flow therein.

10.3.1 Gross Mucosal Morphology

The mucosa of the proximal small intestine is thrown into transversely orientated crescentic folds, the plicae circulares or valves of Kerckring (Junqueira and Carneiro 2005). The formation of such folds may result from differences in the mechanical properties or the basal tone in the component layers of the wall of the intestine, thereby generating residual stress in the innermost portion of the intestinal wall (Gregersen et al. 1997). The existence of such stress is evident when living segments of small intestine are divided either radially or longitudinally, as strips of the wall tend to turn 'inside-out' due to the greater residual compressive stress in the mucosa than in the muscular regions (Gregersen and Kassab 1996). Similar results have been obtained with segments of the guinea pig oesophagus (Assentoft et al. 2000; Lu and Gregersen 2001), the large intestine of the rat (Dou et al. 2002), and the walls of the various tubular components of the biliary tract (Dou et al. 2002).

Plicae circulares are found throughout the small intestine but are more numerous in the jejunum (Junqueira and Carneiro 2005) than in the duodenum (the region of the small intestine with the thickest mucosa and submucosa and which is stiffest in both the longitudinal and radial directions) (Gregersen 2003).

The effect of plicae on fluid dynamics at the mucosal border has not been evaluated but it is likely that radial projecting edges will induce vortices in their rear, provided flow rates are high and the fluid at the periphery of the lumen is of low viscosity, a situation that is only likely in the proximal small intestine. Recent in vivo evidence suggests plicae persist in the face of considerable hydrostatic distension (Knutson et al. 2009).

10.3.2 The Villi

The mucosa of the entire adult human small intestine, with the exception of areas where there are submucosal accumulations of immune cells (Peyer's patches), is covered with villi. Villi are also found in the embryonic colon in a number of vertebrate species at birth (De Santa Barbara et al. 2003) but disappear in human embryos prior to birth (Bell and Williams 1982). It is evident that villi increase the gross surface area of mucosa but, as discussed earlier, there is ongoing debate as to whether this similarly augments digestion and absorption. An alternative view is that villi can move with the flow of digesta, and hence reduce shear forces exerted on the villous mucosa.

The mean height of human duodenal villi, as measured from fresh biopsy material, is around 700 µm (Hasan and Ferguson 1981). The dimensions of villi reported in histological studies may differ from those determined in vivo as it is likely that villous height is altered during histological processing. The size and shape of intestinal villi vary between species (Barry 1976), with age (Ross and Mayhew 1985), and with site, i.e. villus height is greatest in the jejunum and shorter in the duodenum. Magnifying endoscopy indicates that healthy human duodenal villi are of symmetrical finger-shaped configuration (Cammarota et al. 2004). However, human intestinal villi are reported to become leaf shaped when high levels of villous loss are occurring (Creamer 1964).

Each villus contains a thin axial column of longitudinally oriented smooth muscle cells, which is enveloped in a layer of fibroblasts (Güldner et al. 1972). This structure is connected by a network of myofibroblasts (Hosoyamada and Sakai 2007) to a subepithelial network of extracellular fibres (Komuro 1985; Komuro and Hashimoto 1990). The whole structure is thought to provide a degree of circumferential tension which is sufficient to counter any radial volumetric expansion of the villus resulting from the transit of fluid absorbed by the enterocyte into the interstitial fluid (but see below) (Hosoyamada and Sakai 2007). Similarly, the longitudinal muscles are thought to develop a level of longitudinal tension, notably in association with lymphatic channels, so as to counter any axial component of volumetric expansion. The longitudinal smooth muscle cells may also develop cyclic (2–7 Hz) phasic contractile activity that results in piston-like or pendular up-and-down movements of individual villi (Womack et al. 1988b), notably during absorption of nutrients (Womack et al. 1988a). Such activity may also serve to displace absorbed fluids into submucosal vessels and/or induce displacement or stirring of liquids within the paravillous spaces (Mailman et al. 1990). However, there is little experimental evidence for the latter, and experiments with exteriorised segments of canine small intestine (duodenum and ileum) showed no improvement in absorption when villous movements were stimulated (Mailman et al. 1990). Moreover, the frequency and forcefulness of villus contractions are higher in the jejunum than in the ileum (Mailman et al. 1990), whereas in the latter, the absorption of generally lower ambient concentrations of nutrients would presumably be most benefited by activities that augment mixing.

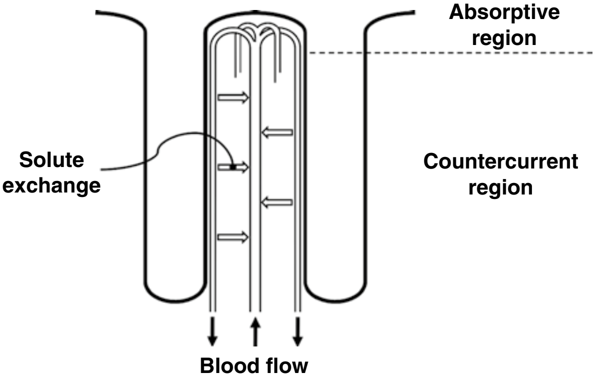
Enterocytes that are capable of producing hydrolytic enzymes (Ugolev 1989) and that possess intramembraneous fluid (Lee 1969), glucose transporters (Kinter and Wilson 1975), and sodium transporters (Hallbäck et al. 1991; Pappenheimer 2001b) are found only in the apical region of the villi. The enterocytes in the lateral epithelium of the villus may only be able to augment uptake by passive absorption, if at all. No absorption is thought to take place from the intervillous crypts at the bases of the intervillous spaces (Kinter and Wilson 1975). It is known that villous enterocytes undergo continual renewal, fresh enterocytes being produced from germinal cells situated at the villous bases and continually being shed from the apex (Marshman et al. 2002) into the overlying mucus layer. Thus the disposition of functions may reflect a villus base-to-tip gradient in enterocyte maturity (Barker et al. 2008). A similar gradient may occur within the adjacent intervillous crypts, i.e. the renewal of cells in the germinal region in the neck of the crypt with increasing maturity towards the crypt base (Barker et al. 2008).

It is conceivable that form drag and turbulent mixing could occur in the intervillous space when fluid digesta flows across the apical surfaces of villi (Leyton 1975). However, it is likely to be reduced when villi become densely packed together. Indeed, given the low Reynolds number of digesta, the low velocity of peristalsis, and the reduction of surface friction by the adherent mucus layer (see below), it could be concluded that little vortical or turbulent flow could be established within a closely packed villous layer. Recent evidence suggests that the residual stress that results from differences in the tone and mechanical properties of components of the intestinal wall (see above), may cause the villi to be subject to circumferential compression. Hence the mucosal layer has been shown to contribute to the viscoelastic creep that occurs when circular segments of small intestine are incised radially (Zhao et al. 2003). However, this finding is complicated by the fact that living villi are reported to swell progressively ex vivo, presumably due to the progressive engorgement of their tissues with absorbed but untransported fluid (see below), and hence to occlude adjacent intervillous spaces in ex vivo preparations (Ryu and Grim 1982).

Recent in vivo studies show there is significant widening of duodenal intervillous spaces when the lumen is distended (Cammarota et al. 2004). Hence, contrary to earlier hypotheses (Ryu and Grim 1982), the thickness of the villous layer may indeed vary during peristalsis causing fluid to be alternately displaced from and drawn into intervillous spaces (see above) aiding absorption (Holzheimer and Winne 1989). However, it is important to note that such findings do not rule out the possibility that longer term alterations in intestinal dimensions, such as a prolonged reduction in basal tone, may also serve to increase the effective thickness of the intervillous fluid space. Under the latter conditions, an additional component of intervillous diffusion resistance from the fluid occupying the space between adjacent villi may have a greater effect in reducing net absorption than does the effect of increasing absorption on the lateral surface of the villi in augmenting it (Holzheimer and Winne 1989). Modelling studies indicate that the interplay between increased diffusion resistance from longer-term augmentation of the depth of the unstirred fluid and the decrease from short-term changes in volume and mixing between adjacent villi may also depend on the permeability of the villous membrane to the nutrient (Winne 1989). If the epithelium is highly permeable to the diffusing substance, then there is an initial period of increased flux immediately after the widening of the intervillous space, but this subsequently declines to below that obtained when the intervillous distance is low.

The orientation of arteriolar and capillary flow along the length of the villous has been postulated to act as a countercurrent multiplier system that maintains apical tissue at a higher osmotic pressure than tissue nearer the base of the villi (Hallbäck et al. 1978) (Fig. 10.2). The two arteriolar vessels that supply each villus run to the tip before branching into capillaries and veins, which run towards the bases. This countercurrent flow allows solutes that are absorbed in the veins to diffuse into the arterioles, i.e. form a countercurrent exchanger. The lengthwise disposition of the vessels causes the concentration difference to increase from the base to the tip of the villus so that a maximal concentration of solutes is maintained at the villus tip, i.e. forms a countercurrent multiplier. Evidence supporting this mechanism comprises an increase in extracellular sodium concentration from the villus base to its tip (Haljamäe et al. 1973), base-to-tip gradation of osmolarity, and cross-diffusion of oxygen (Bustamante et al. 1989). However, the extent of countercurrent exchange may vary with the physicochemical properties of the solute. Thus while H_2, He, CH_4 and

Fig. 10.2 Showing countercurrent exchange system in a villus. Absorption occurs at the villus tip while countercurrent exchange takes place between the central arteriole and the peripheral capillaries and veins maintaining a higher concentration of solutes at the apex. (Bond et al. 1977)



Xe were all absorbed efficiently (99.7, 99.9, 75.6 and 36.0%, respectively), the absorbed fraction that passed into the systemic circulation was lower (16.2, 12.8, 12.0 and 15.8%, respectively) (Bond et al. 1977). This indicates that the countercurrent exchange rates were significantly different, if it is assumed that none of the gases had differentially partitioned in other tissues.

The establishment of a countercurrent gradient with the highest concentrations of a solute being maintained in the tip depends on limitation of absorption from the sides of the villus by dense packing of the villi such as occurs in the dog (Bond et al. 1977). As discussed earlier, in situations where the villi are more widely spaced, such as in the rabbit, or where distension temporarily increases convection in the paravillous space, then inward diffusion can occur along the entire length of the villus so that it becomes equilibrated with the external concentration of the solute. Blood will similarly become equilibrated immediately on entering the villi and no countercurrent system will be established (Bond et al. 1977). It is of interest to note that while there appears to be no countercurrent system in rabbit villi, their rates of absorption are significantly higher than, and the rate of blood flow significantly lower than those in animals that possess a countercurrent system (Bond et al. 1977). Hence countercurrent systems may act to augment absorption of water and of solutes by solvent drag only when villi are closely packed.

As well as influencing water absorption rate and local permeability to small molecular probes such as mannitol (Bijlsma et al. 1995), intestinal countercurrent multiplication may lead to relative retention of absorbed materials and increase the likelihood that they are metabolised locally (Lin et al. 1999).

10.4 Mucins

The gut epithelium is coated from stomach to colon with a layer of mucus. The layer possesses chemical and physical properties that may contribute to the protection of the epithelial cells and the mucosa from penetration by pathogenic microorganisms as well as providing lubrication and mechanical protection of underlying epithelial cells. While the 10-µm thickness of the tightly adherent layer of mucoproteins that constitutes the vascular glycocalyx is unlikely to provide a significant barrier to diffusion of substances with a molecular weight of less than 10,000 (Michel and Curry 1999; Pappenheimer 2001b), the firmly adherent mucin layer and the mobile mucin layer associated with gut epithelia constitute a significant impediment to diffusion, and hence form a significant component of the UWL (Boshi et al. 1996). However, rather than forming a simple diffusion barrier (Peppas et al. 1984), the charged polysaccharide side chains and the hydrophobic areas in the proteinaceous regions of the mucin molecules may interact with and hinder the passage of certain substances (Cone 2009).

10.4.1 The Molecular Structure of Mucins

Mucins have a characteristic general structure formed from protein monomers. Each monomer comprises a flexible protein core with a length of 0.2–0.6 µm and consisting of over 5,000 aminoacid residues. Sequences of protein monomers linked end to end by disulphide bonds form mucin fibres (Carlstedt and Sheehan 1984). Each protein monomer has regions with successive hydrophilic PTS (proline, threonine and serine) rich sites to which numerous oligosaccharide side chains are linked by O-glycosidic bonds. These PTS-rich regions are interspersed with non-glycosylated cysteine-rich hydrophobic regions which each fold into a hydrophobic globule stabilised by internal disulphide bonds (Cone 1999) (Fig. 10.3).

The typical mucin molecule is of linear topography (Fig. 10.3) and is composed principally (80%) of proteoglycans attached to a central protein fibre up to 10 µm in length (Carlstedt and Sheehan 1984; Shogren et al. 1989) giving it a bottle brush configuration with a diameter of around 3–10 nm (Shogren et al. 1989). Each glycan side chain is tipped either by galactose, n-acetyl galactose, fucose, sialic acid or sulphate, the latter two giving the side chain an overall negative charge (Khanvilkar et al. 2001). The composite structure of the mucin fibre, comprising discrete hydrophobic regions interspersed with flexible hydrophilic glycan-laden segments, is sufficiently flexible to allow these regions to associate with similar areas on adjacent mucin or other molecules. Thus mucin fibres are inherently ‘sticky’ (Cone 2009).

Recent work indicates that mucins may be grouped into three broad categories. Firstly, there are the large gel-forming mucins, namely the products of MUC2, MUC5AC, MUC5B and MUC6 genes. The second category comprises the more rigidly constructed membrane-associated mucins, which do not contain cysteine-rich domains and do not form multimers. They are products of the MUC1, MUC3, MUC4 and MUC12 genes. The products of MUC 1 and MUC4 genes are thought to have a role in the protection of membrane surfaces. Of the latter two, the MUC4 product is the larger and is thought to project 4 µm above the surface membrane. The third category includes the MUC7 gene product, which is a small soluble mucin and gives rise to a number of factors that are important in oral processing of food. A number of mucin gene products have not as yet been assigned to one of these categories (Offner and Troxler 2000).

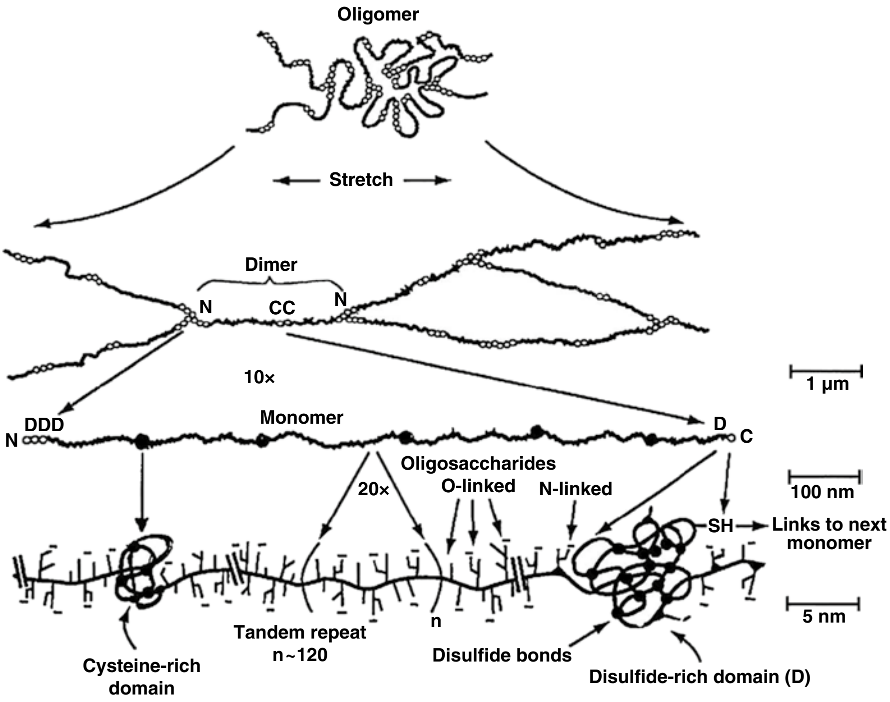


Fig. 10.3 Major biochemical features of gel-forming mucins. Several mucin monomers whose synthesis is directed by MUC genes are shown joined together via disulphide bonds in disulphide rich domains. Individual mucin fibres are shown are densely glycosylated with O- and N-linked glycans. (Cone 1999)

Mucins contain a number of 'impurities', including exfoliated cells and a variety of proteins, e.g. β-lactoglobulin, collagen, albumen and fibrillin. It is not known whether the presence of these entities results from contamination during mucus sampling or from permeation in vivo from extracellular fluid. A further protein which binds to the Fc terminal of gamma globulin FCGBP (Harada et al. 1997) is covalently attached to MUC2 via specific domains (Johansson et al. 2009) and hence is a functional constituent. The presence of this latter element indicates that antibodies can be synthesised and subsequently bound to mucin strands so that an immune defence is intercalated with a mechanical defence against invasion by pathogens.

10.4.2 The Synthesis and Secretion of Mucin

The gastrointestinal mucosa secretes nearly 10 l/day of mucus (Powell 1986). Intestinal mucins are secreted mainly by goblet cells but can also be produced in enterocytes. The synthesis of the monomers that form the protein backbone is encoded by an array of MUC genes but the enormous repertoire of oligosaccharide side chains that can subsequently be attached generates a wide range of structural form, including the generation of mucins with side-chain geometries that may allow them to bind specifically with pathogens and secreted antibodies. The expression of individual MUC genes and the expression of the suite of enzymes associated with the glycosylation of mucins are restricted to certain sites and tissues (Rose and Voynow 2006) presumably to tailor the properties of the resultant mucins according to local conditions and function.

The total thickness of the mucus layer varies regionally (Fig. 10.4) (Atuma et al. 2001; Sarosiek et al. 1991) being thicker in the stomach (50–450 µm) (Copeman et al. 1994; Kerss et al. 1982; Sandzen et al. 1988) and colon (830 µm) (Atuma et al. 2001; Copeman et al. 1994), and thinnest in the jejunum (80 µm) (Atuma et al. 2001) and areas overlying the M cells of Peyer's patches (the sites of microbial and antigen sampling) (Cone 2009; Frey et al. 1996). The luminal boundary of the mucin layer is lipid rich and may limit fluid convection (Cone 2009) and adhesion between adjacent villi.

The thickness of the gastrointestinal mucus layer results from a dynamic balance between the rate of secretion and the rate of erosion by shear and autolysis (Allen and Flemström 2005). Broadly speaking, the mucin layer tends to be thicker in areas that experience high levels of shear, either from forcible contractile activity or from more solid-like digesta (Fig. 10.4). Similarly, it tends to be lower in sites where digesta are more fluid, and where villi may reduce mucosal shear by virtue of their flexibility allowing their tips to move with the flow of digesta.

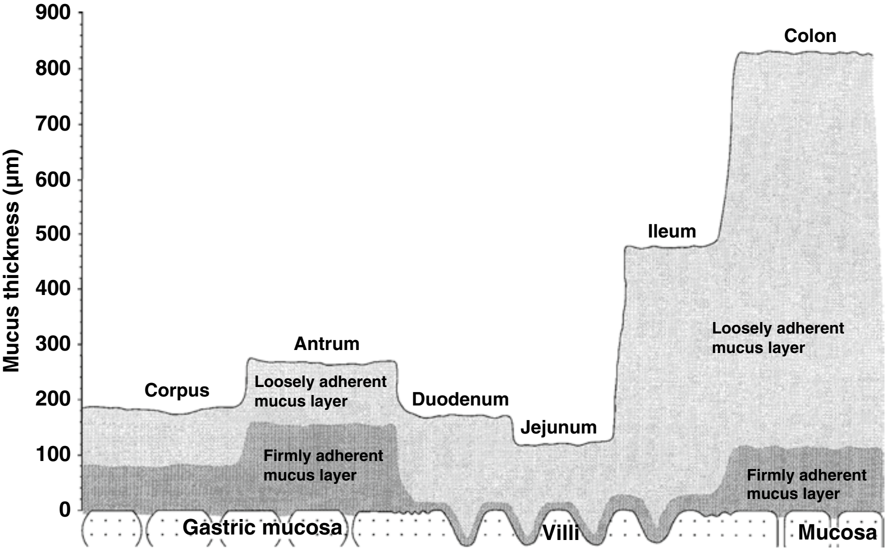


Fig. 10.4 Showing the thickness of the mucus layers in various segments and compartments of the gastrointestinal tract of the rat. (Atuma et al. 2001)

The rate of secretion is also influenced by conditions in the lumen, by local neuronal, immunological and hormonal signalling in the mucosa, and by bioactives from commensal microflora (Deplancke and Gaskins 2001). Thus the total thickness of the mucin layer in the rat duodenum varies according to the acidity of its lumen contents, the local levels of prostaglandins, and the level of vagal stimulation (Akiba et al. 2000). Secretion may also be augmented by mechanical stimuli (Atuma et al. 2001). If such stimuli cause the rate of mucin secretion to outstrip that of intracellular synthesis, the physical characteristics of the secreted mucin and of the mucin layer become more fluid, and hence more susceptible to shear (Akiba et al. 2000). Similarly, mucin secretion may be stimulated by bacterial lipopolysaccharides (Enss et al. 1996).

The high secretion rate (Powell 1986) and fast volumetric expansion of mucin liquid crystals (600-fold expansion of volume in 40 ms) on their release from mucin-secreting cells (Davies and Viney 1998) are thought to be sufficient to prevent larger molecules, such as pepsin, from diffusing inwards from the lumen (Allen and Flemström 2005). This remarkable rate of expansion results from the dense packing of the mucin molecule within storage granules of mucin cells. The mucin molecules in these granules are extensively folded with a high degree of orientational order, i.e. brush interdigitation (Waigh et al. 2002) of their polysaccharide side chains, forming nematic liquid crystals (Davies and Viney 1998; Waigh et al. 2002). The packing and folding of mucins within the granule are aided by high concentrations of calcium ions, which form divalent protein–protein links between adjacent fibres and diffuse away when the granules are liberated into the lumen (Verdugo 1990).

The outer mobile layer of mucins (Atuma et al. 2001; Taylor et al. 2004) appears to move distally over the inner adherent layer once shed. The relative retention time of mucin-adherent polycarbophil particles through a loop of proximal jejunum and a loop of colon exteriorised from anaesthetised rats was around 120 and 80 min, respectively, compared to 70 and 80 min for non-adherent particles (Tirosh and Rubinstein 1998). This indicates that digesta and other particles that adhere to mucins in the small intestine may be retained for longer than particles that remain in the lumen. Conversely, there appears to be no difference in the retention time of particles that adhere to the mucins in the colon than those that remain in the lumen. The difference between the two gut components may reflect the differences in the site, rate and type of mucin synthesis, and the level of interaction with particulate material in digesta. In either case, exfoliated mucins are able to envelop particles regardless of their ability to adhere to mucin (Florey 1962; Gruber et al. 1987; Tirosh and Rubinstein 1998).

The glycocalyceal layer comprises the innermost anchoring point of the adherent mucin layer. The projecting elements of membrane-anchored mucins are densely interspersed with secreted mucins (Frey et al. 1996; Maury et al. 1995) to produce a structure that has greater viscoelasticity and shear resistance than the more superficial mucins in the mobile mucin layer (Taylor et al. 2004).

The adherent or glycocalyceal layer of mucus comprises mucin fibres, which are anchored to the apical surfaces of the epithelial enterocytes via transmembrane domains. Immediately lumenwards to each transmembraneous mucin domain is a

SEA (sea urchin sperm protein, enterokinase and agrin) domain, a protein amino-acid sequence that can undergo rapid lysis in response to mechanical stress without disrupting the membrane (Cone 2009). Hence, these areas provide a plane of cleavage in the extracellular portion of the adherent mucin molecule preventing intramembraneous fracture or other damage to the enterocyte when undue shear stress is applied.

10.4.2.1 Regional Variation of Mucin

The expression of the MUC genes varies both with site and with mucosal cell type. Mucins produced from the MUC2 gene predominate in the gastrointestinal tracts of humans, rats and mice (Gum et al. 1994; Herrmann et al. 1999; Karlsson et al. 1996), and originate only from goblet cells. The MUC3 gene is expressed in both goblet cells and enterocytes (Chang et al. 1994), while MUC4 is expressed in both the small and large intestine (Audie et al. 1993). MUC 5b and MUC6 are expressed in the goblet cells of the colon (De Bolós et al. 1995), and MUC6 in Brunner's glands in the duodenum (Ho et al. 2000). The MUC5AC gene is expressed by the gastric epithelial mucus cells, while the MUC6 gene is expressed by gastric glands (Ho et al. 2000).

The pattern of glycosylation of the protein backbones of mucins varies both regionally and locally. Neutral mucins predominate in the stomach, while acidic mucins predominate in the large intestine (Sheahan and Jervis 1976). Of the acid mucins, there is a greater proportion of sulphonated oligosaccharides in MUC2 mucins from the large intestine than in those from the small intestine, and a greater proportion of sialylated oligosaccharides in MUC2 mucins from the small intestine than from the large intestine (Karlsson et al. 1997). More recently, Matrix assisted laser desorption/ionisation time of flight spectroscopy (MALDI-TOF) and nuclear magnetic resonance spectroscopy (NMR) studies have demonstrated gradients from ileum to rectum of increasing amounts of oligosaccharides containing N-acetylneuraminic acid and of decreasing amounts fucosyl-containing oligosaccharides (Robbe et al. 2003). Given the differing charges on these various moieties, it is likely that they may influence not only the rheological properties but also the permeabilities of mucins to the passage of charged molecules.

Some of the structural differences between intestinal mucin in different segments of the gut may result from 'cross-talk' with differing segmental populations of enteral microflora. It is known from comparisons of the structure of intestinal mucins from germ free with those from normal animals that intestinal microbiota influence the dynamics of mucin synthesis, notably the ratio of neutral to acidic mucins (Deplancke and Gaskins 2001).

10.4.2.2 Glandular Organisation of Mucins

Recent work with stomach mucosa has highlighted the manner in which the production of various types of mucins is coordinated with the secretion of digestive agents

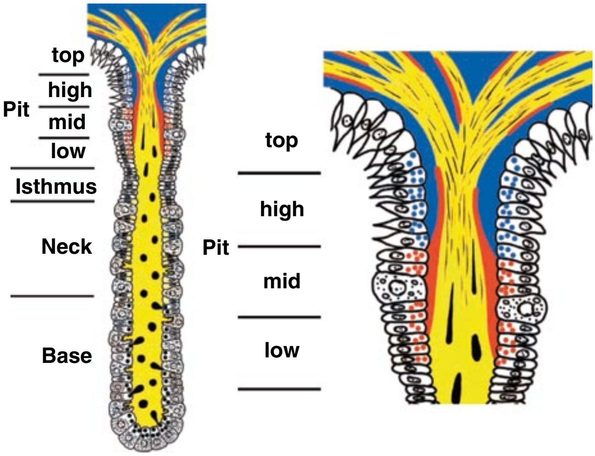


Fig. 10.5 Schematic representation of various immune reactive types of mucin in and around the gastric glands of the rat. In the pit region, the MNC mucin *(yellow)* and sulphated mucin *(red)* form a channel surrounded by RGM11-positive mucin *(blue)*. (Sawaguchi et al. 2002)

by mucosal glands. In the case of the stomach, this allows the integrity of the mucin layer to be maintained and the mucosa to be protected, while potentially destructive digestive secretions are liberated into the lumen. Given the regional variation in the production of mucins, it seems likely that glands in other sites along the gastrointestinal tract will exhibit similar specialism in this regard.

Within the glandular portion of the rat stomach, different mucins are secreted by goblet cells in the neck and the body of the gastric glands, and within the pits that lead to these glands (Fig. 10.5), as well as by cells on the surrounding surface mucosa. The cells in the lowest and middle portions of the pit secrete a sulphonated mucin that reacts specifically with cationic colloidal gold (Yang et al. 1996), while the mucins produced by cells in the more superficial region of the pit react specifically with the monoclonal antibody RGM11 (Ishihara et al. 1993). The cells in the more deeply situated necks of the gastric glands secrete mucous neck cell (MNC) mucin, which specifically reacts with an agglutinin from *Griffonia simplicifolia* (Ihida et al. 1988). High-pressure freeze techniques show that zymogen granules from secretory cells in the submucus glands become enveloped in MNC mucin and have a droplet-like appearance when they are liberated into the lumen of the gland. However, they become progressively elongated as they ascend into the neck and merge with the tracts of MNC mucin, which at this point are surrounded by laminae formed from the other mucin types that are produced in the pit (Sawaguchi et al. 2002). Hence the mucus layer superficial to the gland comprises alternating laminar arrays of the three types of mucus, the MNC layer having undergone a degree of proteolytic degradation (Fig. 10.5) (Sawaguchi et al. 2002). These findings of encapsulation of digestive secretions by mucins are in accord with results obtained with the application of congo red and acridine dyes, which suggest that secreted acid transits from the gastric crypts across the surface mucus layer into the luminal bulk solution at restricted sites (Holm and Flemström 1990), in discrete packages (Bhaskar et al. 1992; Johansson et al. 2000), and with reports of mucin channels within the lumen of gastric glands (Allen 1978; Johansson et al. 2000). Thus the local disposition of mucins prevents local denaturation and autolysis of the mucosa.

The mucin layer in the mouse colon consists mainly of MUC2 mucin (Johansson et al. 2008), an inner 50-µm layer containing a high concentration of MUC2 mucin, which is firmly attached to the epithelium, and an outer loose layer, which is less firmly attached and has a fourfold lower concentration of MUC2 mucin (Johansson et al. 2008). The gross chemical properties of the two layers also differ, the firmly attached layer being insoluble in guanidium chloride as a result of covalent cross-linking between constituent monomers (Axelsson et al. 1998; Herrmann et al. 1999). As in the stomach, the colonic mucin layer comprises a laminated rather than a homogenous structure, in this case, comprising alternating layers of sialylated and sulphonated mucins (Matsuo et al. 1997).

We can find no reports of a lamellar architecture in the mucin layer of the small intestine. This may be a consequence of its villous architecture and the relatively thin mucus layer (Atuma et al. 2001). However, given the evidence of mucin layering in regions of the gastrointestinal tract with simpler mucosal morphology, it seems reasonable to conclude that the mucin layer is inhomogeneous and that such inhomogeneity may impart local differences in rheological properties, and in immunological and microbiological reactivity. Hence a degree of caution is required in interpreting work describing the macro-rheological properties and overall permeability of bulked mucin.

10.4.3 The Physical Properties of Mucins

Aside from their role in resisting penetration by pathogens, it seems likely that intestinal mucins are variously adapted to physically protect the mucosa from abrasion. If this is so, we may expect to see local and regional variation in the thickness and physical properties of mucin according to the differing physical properties and flow of digesta.

While the thickness of the mucosal layer in a particular segment of gut varies broadly with the rheological properties of the contained digesta, there are grounds for suspecting that the chemical, and hence the rheological properties of mucins may also vary within the site at which they are secreted. For instance, colonic mucins have three predominant types of O-orthoglycan core structure, each with a distinctive pattern of terminal groups (Rose and Voynow 2006). These groups variously influence the charge and hydrophobicity of the mucin, sialic acid and sulphate moieties, imparting a negative charge, while fucose moieties impart hydrophobicity. Such variation has the potential to alter the physical properties of the mucin, although sufficient quantities of purified mucins with particular core groups are not yet available for characterisation (Rose and Voynow 2006). If the increase in charge does influence the rheological properties of the mucin (Tsai et al. 1995), it seems likely that both the thickness and rheology of the mucin layer may also change along the base-to-tip axis of the small intestinal villus (Specian and Oliver 1991; Vecchi et al. 1987). Thus, as goblet cells mature and migrate to the villous tip, they continue to renew and discharge mucin granules (Chambraud et al. 1989), but their mucins become increasingly sialylated and undergo further N- and O-acylation (Specian and Oliver 1991).

Early quantitative work on the physical properties of bulked mucus was complicated by its variable stability, native small intestinal mucus being readily disrupted by acid, bile and detergents, while colonic mucus was resistant to these treatments (Sellers et al. 1991). However, the instability of small intestinal mucus was later shown to result from the presence of insoluble materials, such as exfoliated cells, rather than from differences in the rheological properties of the contained mucins. The development of techniques for removing these elements paved the way for physical characterisation of bulked mucins.

We describe below work documenting the permeability, tribology and rheology of bulked mucins.

10.4.3.1 The Permeability of Mucins

There is increasing evidence that the mucin layer functions as a barrier, which is interposed between the lumen and submucosa, and that in conjunction with solvent drag (Pappenheimer 2001b) generated by absorbing enterocytes, it limits the passage of solutes of larger molecular size and particular molecular characteristics, as well as excluding larger particles and most enteral microflora. By acting as a particulate and selective molecular sieve, it may modify and to some extent, stabilise the aqueous environment immediately adjacent to the apical plasma membrane of the enterocyte, in effect forming a subsidiary compartment to the lumen.

While the presence of the mucin layer undoubtedly contributes to the diffusion barrier (Levitt et al. 1988; Thomson and Dietschy 1977), it does not function in a manner that is strictly analogous to a layer of unstirred water (Peppas et al. 1984). In particular, the permeability of the mucin layer varies inversely with solute molecular size and is generally reduced with respect to that of a layer of water of similar thickness (Desai et al. 1992), some workers reporting a fivefold retardation with respect to water (Nicholas et al. 1991; Turner et al. 1985). Also, plots of the ratio of mucous to aqueous diffusion coefficients obtained with probes of different molecular size show that mucin permeability is much lower for probes with a molecular size over 68 kDa, i.e. bovine serum albumen (Desai et al. 1992). However, some of these differences may not result entirely from molecular size. Mucin permeability is also influenced by the net charge and hydrophobicity of the diffusing molecule. The diffusion of short-chain fatty acids (Smith et al. 1986) and aminoglycosides (Niibuchi et al. 1986) is significantly impaired with respect to water. While most soluble globular proteins diffuse readily through mucin gels (Olmsted et al. 2001; Saltzman et al. 1994), the diffusion rates of secreted antibodies, such as IgM, are relatively reduced as a result of their large pentameric form (Cone 2009) and the presence of a slightly mucophilic secretory component (Corthesy 2003; Olmsted et al. 2001). Indeed, the latter may be a desirable feature in aiding antibodies to be sequestered close to their site of secretion and in the 'trapping' of pathogens (Cone 2009).

Virus capsid proteins of up to 55 nm are able to diffuse freely through mucus (Olmsted et al. 2001), while hydrophobic particles of the same size, such as polystyrene nanospheres, adhere firmly to the mucin and become enveloped in mucin strands (Cone 2009). The diffusion of small hydrophobic molecules, such as testosterone, through mucin is similarly retarded (Matthes et al. 1992), the diffusion constant decreasing in proportion to the ratio of non-polar to polar diffusion coefficients, presumably according to the extent to which they bind with or partition into the hydrophobic regions of the mucin molecules (Cone 2009). However, it is noteworthy that multilayer liposomes around 200 nm in size have been shown to penetrate the mucus layer of the small intestine (Desai et al. 1992).

The data regarding the effect of molecular size on diffusion through mucus are in accord with a dimensional model based on the scaling of physical obstructions, which predicts the average mucin pore size to be around 100 nm (Amsden 1998, 1999; Amsden and Turner 1999), and with similar spacing between mucin fibres to that found in electron micrographs of ex vivo ovulatory cervical mucin fixed in gluteraldehyde (Cone 2009). However, polystyrene particles of up to 400 nm are able to diffuse through mucus when their surfaces have been densely coated with PEG (see below), the diffusion rate of 400-nm PEG-coated spheres being only fourfold slower than that in water (Lai et al. 2009). This suggests that native mucin fibres may aggregate under certain circumstances to form cables and create larger voids, which allow larger particles to transit (Cone 2009). Alternatively, weak non-covalent interactions between adjacent mucin fibres may slip and allow larger structures to distend the voids (Cu and Saltzman 2009).

As noted earlier, the permeability of a gel also varies according to the net surface charge of the permeating molecule. The presence of a large number of polyvalent cationic groups may cause the molecule to bind avidly to the negatively charged oligosaccharide side chains of mucins (Cone 2009). Thus nanoparticles coated with chitosan (Kas 1997) bind tightly to mucin strands and can collapse the gel (Dawson et al. 2003). Conversely, highly anionic proteins may undergo repulsion from like charges on the oligosaccharide side chains of mucin preventing them from entering and diffusing through the gel (Cone 2009). It is noteworthy that both these effects result from variation in net molecular charge. Thus a number of proteins with a high density of anionic and cationic groups that are distributed evenly over the surface of the molecule, but with overall electroneutrality, can diffuse unhindered though the gel (Cone 1999). It is thought that similar characteristics allow certain virus particles to readily transit the mucin layer and infect the host. Such viruses are generally small, i.e. 30–200 nm, are ‘charge-neutral’ overall, and possess capsid proteins with high densities of anionic and cationic groups (Cone 2009).

Recent evidence suggests that particles of around 100 nm may have greater mobility in acidic environments, e.g. pH 2, where the mucins become extensively cross-linked, than in more alkaline environments, e.g. pH 6, where the mucins are in solution. Micro-rheological measurements indicate that in an acidic environment, porcine gastric mucin forms a heterogeneous gel with a wide range of pore sizes, some of which are sufficient to allow 100–200-nm particles to move within the gel.

However, in an alkaline environment, the same mucin forms a sol, which offers uniform resistance to diffusion particles of this size (Celli et al. 2005).

Adherent mucin forms a layer up to 500-nm thick over the tips of microvilli on the apical surfaces of intestinal enterocytes (Ito 1974; Maury et al. 1995). Though thin in comparison to the total thickness of the mucin layer, these adherent mucins form an effective size exclusion barrier, which prevents viruses and bacteria from gaining direct contact with the apical membrane of the enterocytes and also impedes their access to the spaces between adjacent microvilli (Amerongen et al. 1991; Neutra et al. 1987). Studies using particles of various defined sizes and coated with the B subunit of cholera toxin, a ligand that specifically binds to a receptor in the underlying plasma membrane, indicate that the functional pore size of the brush border glycocalyx lies between 7.4–28.8 nm (Frey et al. 1996).

The glycocalyceal layer that covers the mucosal M cells of Peyer's patches, submucosal aggregations of lymphoid tissue in the small intestine, is thought to be reduced in thickness so as to allow for efficient antigen sampling and transcytosis (Neutra et al. 1995, 1987; Savidge et al. 1994). While the brush borders of the M cells lack an organised adherent mucin layer, their apical surfaces are coated with an abundance of terminally glycosylated glycoconjugates, which together form a 20–30-nm layer, sufficient to prevent mucosal access of particles of 1 µm in diameter (Frey et al. 1996).

10.4.3.2 Lubrication by Mucins (Tribology)

Mucins are weak non-Newtonian thixotropic gels that behave as elastic solids under low shear but deform under high shear, and are able to rapidly regain their structure after disruption (Lai et al. 2009). Such properties are well suited to lubricate mucosa during the transit of intestinal contents by peristalsis and other propulsive movements, given that viscid digesta progressing by plug flow will induce high shear between the digesta plug and the gut wall (Lentle and Janssen 2008). The pseudoplastic nature of mucin promotes slippage immediately adjacent to the digesta plug where the high shear rate reduces the apparent viscosity of the mucin. Further, the stratified nature of the intestinal mucin layer with an adherent layer surmounted by a more mobile superficial layer may augment lubrication by forming a slippage plane between the two layers when shear stress is applied (Cone 2009). The ability of mucins to adhere to contained particulate matter (Andrews et al. 2009; Cone 2009) allows a durable lubricating layer to be established on the outer surface of food particles or digesta boluses, which can facilitate their passage, even through segments whose lining mucosa secretes little or no mucin, such as the oesophagus and anal canal (Bongaerts et al. 2007).

Recent work indicates that salivary mucins may also exhibit significant extensional flow as a result of their molecular elasticity; a property that may further promote lubrication as well as promoting surface adhesion and binding of the particulate components of the bolus (Haward et al. 2010).

Lubrication by Absorbed Mucins The molecular structure of mucins and other proteins secreted by intestinal glands (Levine 1993) imparts amphiphilic or hydrophilic properties which promote their adsorption onto any surface, including those of digesta particles, the intestinal epithelium (Andrews et al. 2009; Yakubov et al. 2007), and the glycocalyceal layer of intestinal mucus. Once absorbed, the mucins form a composite entangled structure (Yakubov et al. 2009) which can provide a protective barrier for non-secretory epithelia as well as direct ongoing lubrication of the surfaces of particulate matter and boluses.

As noted earlier, the readiness with which mucins are absorbed onto surfaces is thought to result from the inherent flexibility of the mucin molecule, which allows it to approach and conform to the contours of the surface (Cone 2009). This close approach allows a range of interactions to develop between the component molecules of the two surfaces, which includes chemical linkage via ionic, covalent, hydrogen and Van der Waals bonds, and the development of physical forces from wetting energies, the interdiffusion of polymer chains across interfaces and the physical interlocking of irregularities on the two surfaces (Smart 2005).

The absorption of mucins onto soft surfaces, such as polydimethylsiloxane (PDMS), brings about a long-lasting reduction in the coefficient of friction, which exceeds by an order of magnitude that which would be obtained from an interposing layer of water (Bongaerts et al. 2007). A similar significant reduction in friction occurs when mucins are absorbed onto ex vivo samples of tongue mucosa (Ranc et al. 2006).

The means by which the absorbed mucins provide lubrication is not completely understood. Oral friction can be described as a boundary or mixed friction regime, which implies that the bolus and mucosal surfaces are partially in contact with one another (Dresselhuis et al. 2008). Given that the shear rates at the mucosal interfaces of gut components are likely to be low enough to prevent the permanent entrainment of a sufficient quantity of the liquid phase of digesta to provide hydrodynamic or hydrostatic lubrication, it remains to be determined whether the absorbed mucins alone are sufficient to provide boundary lubrication, and if so, by what means this may occur.

One hypothesis regarding the lubricating properties of mucins is that entrapped counterions generate electrostatic repulsion and act in a similar manner to ball bearings (Raviv and Klein 2002). However, the validity of this hypothesis is called into question by the recent finding that a rise in pH increases the charge density of porcine gastric mucin but does not influence its lubricating properties (Lee et al. 2005). The current view is that the tenfold reduction in friction coefficient that occurs when mucin is absorbed onto rough hydrophobic surfaces (peak-to-valley differences of 27 μm) results from 'viscous boundary lubrication' (Yakubov et al. 2009). This mechanism results from the accumulation of a bilayer comprising a more firmly adherent inner, which increases in thickness with higher local concentrations of mucin, and a more easily desorbed outer layer. Thus, at a given sliding speed, the thicker the absorbed film, the lower the local shear rate and the lower the boundary friction coefficient (Yakubov et al. 2009). However, it is noteworthy that this type of lubrication only occurs at sliding speeds of around 1 mm/s (Yakubov et al. 2009), which may lie below the transit rate of digesta.

The establishment of liquid crystallinity in the absorbed layer of mucins may also provide lubrication. Commercial thermotrophic monomer liquid crystals and dispersions of monomer liquid crystals in polymers are known to provide excellent lubrication (Carrión et al. 2009). Differential scanning calorimetric studies indicate that the critical concentration of crude porcine gastric mucin necessary to establish a liquid crystalline state is in the region of 24–28% w/w (Davies and Viney 1998). More recent studies, using a combination of small-angle neutron scattering, light scattering and rheology, indicate that the overlap of the bottle brush-like areas of porcine gastric mucin occurs at a concentration around 7.1 mg/ml, and that a load-dependent anisotropy, indicating the formation of an entangled polydomain liquid crystalline structure, occurs at concentrations above 21 mg/ml. Further, an entangled liquid crystalline structure is formed at concentrations around 479 mg/ml, which is the concentration that occurs in the storage granules of mucin-producing cells (Waigh et al. 2002). It is important to note that the local concentration of solids in mucins may be augmented by absorption of water from mucinous secretions onto dry porous surfaces of incoming particles of ingesta, and hence admixture of mucins with incoming boluses may promote the formation of liquid crystalline structures.

10.4.3.3 Protection from Shear by Mucins (Rheology)

Recent evidence suggests that the layer of mucins adjacent to the intestinal epithelium is able to absorb large shear forces before the forces propagate into the SEA domains (Cone 2009) of the adherent mucin layer and initiate cleavage. It also indicates that evolutionary specialisation of the component mucins of the mobile layer has taken place with regard to their physical behaviour under local lumen conditions. It is likely that the differing properties of mucins at various sites in the gut mucosa reflect differences in the expression and configuration of the various MUC and glycosylation genes at those sites. It is possible that such differing expression is cued directly by shear forces promoting inflammatory or genetic changes in mucosal gene expression.

The Absorption of Shear Forces by Mucins Mucins differ from strong gels in that they do not rupture when exposed to excessive strain, but differ from weak gels in that they have greater resistance to strain (Taylor et al. 2003). Recent rheological studies on porcine gastric mucous applying either small deformations or cyclic deformations that were large enough to induce the breakdown of the gel have shed light on the mechanisms that underlie this behaviour (Taylor et al. 2003).

These researchers showed that gastric mucin tends to flow rather than rupture (G″ tending to increasingly exceed G′) when its breakdown stress is exceeded (Fig. 10.6) and to gradually recover its ‘solid-like’ properties as the shear stress is reduced. The cyclic patterns of change in solid-like (G′) and liquid-like (G″) properties of the gel, before and following its breakdown, are symmetrical at higher

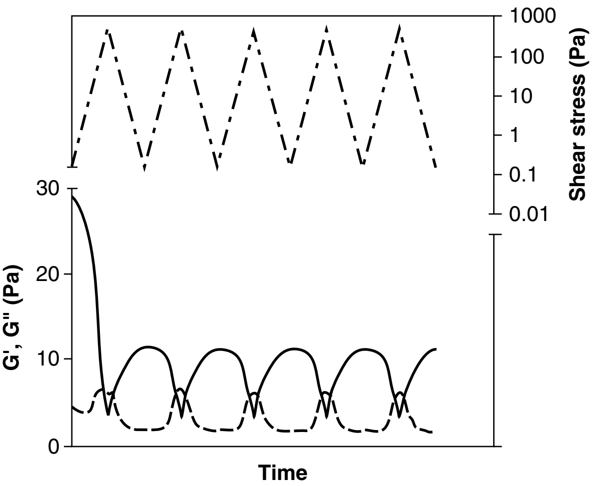


Fig. 10.6 Rheological behaviour of porcine gastric mucus in response to stress-induced breakdown. The applied stress *(top)* was repeatedly swept up to at least twice the breakdown stress and then swept down again. G′ *(solid)* and G″ *(dashed)* were measured at a frequency of 1 Hz. Breakdown was considered as the point at which G″ became dominant over G′. (Taylor et al. 2003)

frequencies of oscillation (1 Hz and above) (Fig. 10.6). Such behaviour is consistent with the formation of a structure in which the component mucin chains are linked by non-covalently associated regions, with more regions on more chains becoming involved as stress is reduced. However, the cyclic patterns of change in solid-like and liquid-like properties of the gel, before and following its breakdown, become asymmetrical at lower frequencies of oscillation. In this case, a series of subsidiary peaks in G′ develop prior to the point of gel breakdown. This behaviour indicates that the solid-like characteristics of the mucin gel result from a range of non-covalent interactions between different portions of adjacent molecular chains, and that these interactions vary in elastic strength. Hence a heterogeneous and dynamic bonding structure between adjacent chains in mucin gels imparts physical characteristics that differ according to the rate at which strain is applied. Rapid deformation induces ‘stress hardening’ of the gel with maximal stress at breakdown, while slower deformation allows time for the strength of these elastic associations to undergo rearrangement and form weaker associations (Taylor et al. 2003).

Such behaviour is well suited to the transit of digesta, allowing the gel to be disrupted and the superficial mucosal layer to flow with the bolus when shear rates are lower and less likely to transmit shear into the mucosa, but to resist disruption more strongly when shear rates are higher. Hence mucin gels that exhibit ‘stress hardening’ may be better able to resist deformation at higher rates of shear such as may occur when a sharp particle is protruding from a bolus towards the gut wall. The hardening of the mucin gel around the sharp particle may serve to prevent it from damaging the underlying mucosa. Although this behaviour means the mucus layer must absorb more energy, rheological analyses indicate that this energy is not stored in the elastic components of the mucin gel (Taylor et al. 2005) and thus cannot be mechanically transmitted from the mucus layer to the underlying mucosa. Rather, it is hypothesised that the energy is absorbed in the changes in the conformation of the entangled molecules or in the extent of their packing (Taylor et al. 2005).

Such capacity to dissipate shear force distinguishes intestinal mucus from the similar cell-bound polysaccharides that form the vascular endothelial glycocalyx. The latter offers significant hydrodynamic resistance to flow (Pries et al. 1997) transmitting shear stress to the endothelial plasma membrane in a graded fashion and initiating the generation of vasodilatory substances via G proteins (Pries et al. 2000).

While mechanisms appear to exist that enable applied shear forces to be absorbed in mucus gels by means other than elastic storage, it is important to note that the local molecular environment can influence the storage of elastic energy, at least in mixed gastric mucus. Hence at pH 2 there is considerable elastic recovery of gel from deformation (creep recovery) but at pH 6 there is insufficient elasticity to enable any such recovery to be identified (Celli et al. 2007). This behaviour may allow gastric mucins in the regions around gastric crypts, where HCl is secreted, to offer greater resistance to mechanical deformation and hence to the intrusion of acid into the mucosa. Salivary mucin demonstrates a similar sensitivity to pH, i.e. its apparent viscosity increases with a lowering of pH and reaches a maximum at pH 4.2 under conditions of low ionic strength (Veerman et al. 1989). Hence the apparent viscosity of mucin surrounding a bolus may increase on entry of the bolus into the gastric lumen; a process that may preserve bolus integrity and allow the action of salivary amylase to continue in the interior.

The molecular events that underlie these changes are beginning to be understood. Results from studies using atomic force microscopy (AFM) (Hong et al. 2005) support the original model (Cao et al. 1999) which proposes that aggregation of mucin molecules to form a gel involves hydrophobic and electrostatic interactions between portions of the mucin molecule that have little no glycosylation. These studies showed that when 50% of the ortho-linked oligosaccharides of mucins had been removed, the protein backbones spontaneously folded into compact globules. Hence the rigid extended fibre-like structures in the mucin molecule are maintained by the oligosaccharides. However, the AFM studies also showed that both deglycosylated and intact porcine gastric mucins had a tendency to aggregate into clusters at pH levels below 5, and thus the association between intact mucin oligomers resulted from the interaction of regions of the mucin back bone that did not bear oligosaccharides (Hong et al. 2005). In view of the concordance of the critical aggregation pH (4.0) obtained from AFM (Hong et al. 2005) and from dynamic light-scattering studies (Cao et al. 1999), Hong and coworkers hypothesised that mucins aggregated at low pH due to the association of the hydrophobic cysteine-rich portions of their backbones. They further suggested that this aggregation resulted from the cysteine-rich regions being made more accessible at low pH, the excess hydrogen ions causing unfolding by influencing constituent non-polar amino acid residues, i.e. glutamate and aspartate.

Differences in the Mechanical Behaviour of the Mobile and the Adherent Mucin Layers Mucus from the mobile layer of the stomach contains a lower concentration of mucin than that from the adherent layer, it exhibits a different lectin-binding repertoire to that of adherent mucus, it exhibits greater reactivity to an antibody raised against human MUC5AC, and it is more susceptible to digestion by pepsin (Taylor et al. 2004). These differences in chemical composition suggest that the mobile mucus layer is not a simple dilution or digestion product of the adherent layer (Taylor et al. 2004), and a body of work supports this hypothesis. Genetic studies show that higher-density mucus is secreted by surface mucus cells and lower-density mucus by mucus glands (Nordman et al. 2002). Biophysical studies show clear differences in the rheological properties of the two layers (Cone 2009; Taylor et al. 2004). While the mucus from both the superficial and adherent layers of porcine gastric mucin behave as true gels, i.e. G′ greater than G″ and phase angles <45°, the adherent layer is relatively shear resistant and the mobile layer is relatively shear compliant (Fig. 10.7) (Taylor et al. 2004). Further, the mobile gel layer breaks down to a viscous liquid at low shear stresses while the adherent gel is stable to shear stresses 100 times greater. Both types of gel rapidly regain their elastic properties when shear ceases.

These differences in mechanical behaviour may reflect differences in gene products. Hence the multimeric gel-forming mucins, such as the product of the MUC5B gene, are likely to contribute to the superficial mucin layer, while membrane-binding

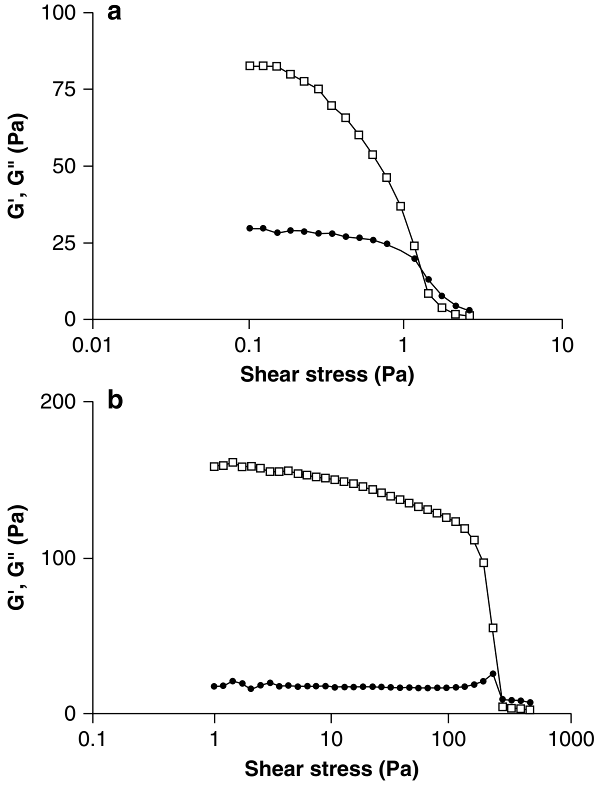


Fig. 10.7 Rheological measurements for a stress sweep of mobile (**a**) and adherent (**b**) porcine gastric mucins. G′ (□) consistently exceeds G″ (●) for both samples indicating that elastic behaviour dominates over viscous. However, G′ is markedly higher for the adherent mucin and breakdown only occurs at much higher stresses. (Taylor et al. 2004)

monomers that are products of MUC 1 and MUC4 genes are likely to contribute to the adherent layer (Offner and Troxler 2000).

Alterations in Mucin Rheology with Local Mucosal Environment As noted earlier, the local pH has a significant influence on the rheological properties of gastric mucin. At pH 6, the loss modulus is higher than the elastic modulus and the phase angle exceeds 45°, i.e. the mucus behaves more like a liquid. Conversely, at pH values ≤4, the elastic modulus is higher than the loss modulus and the phase angle is below 45° indicating that the mucin molecules behave more like a solid, i.e. are gelled (Fig. 10.8) (Celli et al. 2007). This effect has important connotations regarding the secretion and location of mucins and secretory products adjacent to the mucosa. The restriction of outward diffusion of protons from the gastric lumen to the gut wall by the mucus layer is thought to create a pH gradient across it, the pH being higher at the mucus membrane and lower in the lumen (Rees and Turnberg 1982). The maintenance of a higher pH adjacent to the mucosa is thought to protect

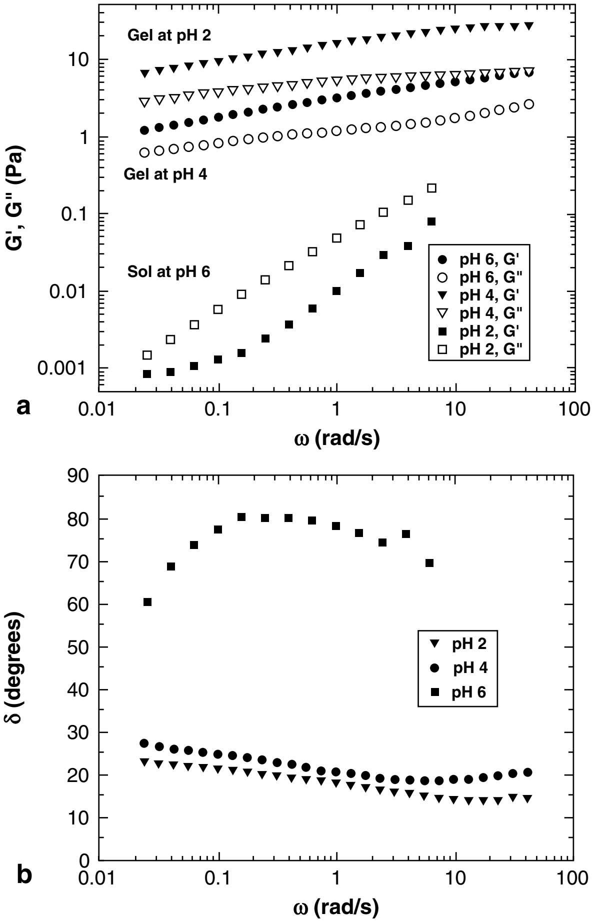


Fig. 10.8 Rheology of porcine gastric mucin under various pH conditions in the linear viscoelastic region. The samples display gel characteristics at pH 2 and 4, but a solution appears to have formed at pH 6. (Celli et al. 2007)

the constituent cells and would also allow gastric mucins secreted by goblet cells to be maintained in a fluid state immediately following their secretion, thereby aiding their transit to the surface. Conversely, secretion of hydrochloric acid would restrict the local flow of mucins and protect the adjacent mucosa. However, these changes would cause the plane of slippage within the mucin layer during propulsion of a bolus to move closer to the mucosa.

At all events, the superficial hardening of gastric mucin is likely to restrict the intrusion of acidic chyme into the mucin layer. When a jet of watery fluid impacts on a viscous non-Newtonian solution of high molecular weight polysaccharides, it permeates the latter in a randomly branched series of 'viscous fingers' (Nittmann et al. 1985). This fractal patterning is thought to result from the random orientation of the polymer chains therein. A similar fractal pattern is generated when a jet of dilute hydrochloric acid impacts on an alkaline solution of gastric mucin, but penetration and branching are reduced when the pH of the mucin is below 4 (Bhaskar et al. 1992), the higher local pH causing the mucin to become more viscous (Bhaskar et al. 1992; Johansson et al. 2000).

These findings raise the possibility that the extent of viscous fingering may be modulated by the rheological behaviour of mucins, which vary regionally and locally due to the dynamics of their synthesis, i.e. according to their genetics and subsequent side-chain modification (see the earlier section on regional variation in mucins). For example, the secretion of a cylinder of acid-hardening mucin within the gastric crypts may direct the flow of hydrochloric acid containing secretions to the surface and prevent it from impinging on the surrounding mucosa.

Given the presence of a number of differently genetically coded and structured mucins at various sites in the gastrointestinal tract, it is possible that such behaviour may occur in other segments of the gut with hardening of mucins at appropriate pH. Mucin preparations generally behave as solutions at very high salt concentrations, i.e. 200 mM. This phenomenon is thought to result from ionic screening of electrostatic interactions reducing the degrees of cross-linking and entanglement between adjacent mucin chains as well as causing individual molecules of mucin to adopt a less-extended configuration (Celli et al. 2007). Consequently, the local secretion of sodium and chloride ions within the colonic crypts may help to maintain locally secreted mucins in the fluid state preventing crypt occlusion and allowing the convective flow of water to the base of the colonic crypt for absorption (Pedley and Naftalin 1993). Similar effects may occur in small intestinal mucosa.

The dynamics of cellular turnover in small intestinal villous mucosa, i.e. cells transiting from the germinal region at the villus base to the tip of the villus before exfoliating (Barker et al. 2008; Marshman et al. 2002), may also influence the pattern of mucus secretion. The functional maturity of the enterocyte appears to increase along the length of the villus as indicated by the restriction of hydrolytic enzymes (Ugolev 1989), glucose transporters (Kinter and Wilson 1975), apical fluid (Lee 1969) and sodium transporters (Hallbäck et al. 1991; Pappenheimer 2001b) to those enterocytes in the villous tip. Similarly, there appears to be an increase in tight junction integrity and a corresponding decrease in intercellular pore size (Gumbiner 1987; Marcial et al. 1984) towards the villous tip. Therefore, it seems likely that the 'maturity' of mucins that are secreted by the enterocyte, and hence the thickness and rheological properties of the adherent mucin layer, may similarly vary along the length of the villous. This may have important consequences in respect of absorption via the paravillous spaces, but as yet, little work has been done in exploring this hypothesis.

10.4.4 Interactions with Enteral Microflora

The colonic microflora can be viewed as the digestive organ of the hindgut (Minekus et al. 1999) forming what amounts to a biofilm on the surfaces of digesta particles and on the mucin components of the intestinal wall, and bringing about the fermentative digestion of material that is not amenable to enzymatic digestion in the stomach and small intestine, thereby accessing additional nutrients (Hume 1999). Therefore, it is relevant to consider the physical dynamics that govern the function of this ancillary 'organ'. Recent evidence indicates that the species composition of the guilds of commensal microflora that constitute the particulate and mucin biofilms differs (Macfarlane and Macfarlane 2004), as must their population dynamics. The continued residence of microbial species that colonise particles is dependent upon the relationship between the residence time of the particles and the microbial generation time and/or ability to disseminate to fresh particles. Conversely, the continued residence of species that colonise the mucin layer depends upon their ability to adhere to the layer while manipulating the host to provide appropriate nutrients and to tolerate their presence. It is increasingly apparent, at least in the colon, that the latter species are largely limited to the outer limits of the mucin layer (Swidsinski et al. 2007a, b) in areas other than immune sampling points, such as Peyer's patches.

10.4.4.1 Binding with Mucins

The ability of the mucins to limit the passage of commensal bacteria from the lumen to the mucosa is evident from: studies of the binding of individual species of enteral microflora to mucin (Kirjavainen et al. 1998; Ouwehand et al. 1999; Rojas et al. 2002); studies using fluorescent in situ hybridisation (FISH) showing that the bulk of microbial species are confined to the outermost region of the mucin layer (Kleessen and Blaut 2007; Poulsen et al. 1994; Schultsz et al. 1999; Van der Waaij 2003); and studies showing that the inner region of the colonic MUC2 mucus layer is devoid of bacteria (Johansson et al. 2008).

The characteristics of intestinal mucins have other effects on the residence and transit of enteral microbiota (Velez et al. 2007). A number of species of commensal microflora that benefit the host are known to bind to (Velez et al. 2007) and utilise intestinal mucins as a food source (Bongaerts et al. 2005; Corfield et al. 2002; Hoskins and Boulding 1981). For instance, the thickness of mucin layer is relatively increased in the gut of abiotic mice, presumably from the lack of mucinolysis by enteric microflora (Deplancke and Gaskins 2001). A number of species of commensal microflora are capable of inducing changes in the glycosylation mechanism of mucin-producing cells of the host causing them to produce mucins that are more favourable substrates for their growth (Bry et al. 1996; Hooper and Gordon 2001). Presumably, this induction occurs via transmucosal cross-talk with the host (Deplancke and Gaskins 2001) by means of soluble products (Hooper et al. 1999).

10.4.4.2 Physical Limitation by Mucins

Pathogenic species are generally able to bind directly to mucosal cell surfaces, subvert cell structure, and invade the host (Isberg and Van Nhieu 1994). Such direct cellular adherence may be prevented by the production of mucins that bind to and prevent the passage of pathogens (Mack et al. 1999). Hence the variability in the pattern of mucin glycan synthesis in response to the presence of microbiota may be viewed as proof of a capacity to generate the necessary structural diversity to incapacitate pathogens, either by binding to them directly or by accommodating immunoglobulins that react with the pathogen (Johansson et al. 2009). The extent to which such chemical changes influence the local rheological characteristics of the mucin is not known.

The mobile and adherent mucin layers also constitute a physical barrier which impedes the transit of particular microflora through the mucosa and thus may help to prevent the establishment of pathogens. While the interfibre spacing of mucin strands may not be small enough to physically impede the passage of viruses smaller than 100 nm (Cone 2009), it is sufficient to physically delay the passage of larger viruses, e.g. the herpes simplex virus (180 nm) (Olmsted et al. 2001). Similarly, evidence suggests that the rheological properties of intestinal mucin impede the motility of certain bacteria (Young 2006). In particular, in vitro experiments indicate that short cocci are able to navigate material of low viscosity but are trapped by material of higher viscosity, while long, rod-shaped bacteria and those with a spiral or curly form are able to navigate both low and high viscosity material (Greenberg and Canale-Parola 1977; Schneider and Doetsch 1974; Swidsinski et al. 2007b; Young 2006). However, these authors did not investigate the effects of the non-Newtonian behaviour of mucins on bacterial motility. Judging by their superficial location within the mucus layer, the motility of rod-shaped peritrichous bacterial species may be restricted to a greater extent than those with helical motility. Studies of bacterial distributions in specimens of mucosa from the proximal colon of the mouse (Fig. 10.9) indicate that, while some bacteria are able to gain access to the crypt bases, the bulk of bacteria are confined to the enteral surface of the mucin layer. Only bacteria with long curly rods and filaments were able to navigate the mucus layer and make direct contact with the colonic mucosa (Swidsinski et al. 2007b).

The mobile mucin layer is known to ‘flow’ distally at a significant rate, to be digested by pepsin, and to disperse in water (Tirosh and Rubinstein 1998). A number

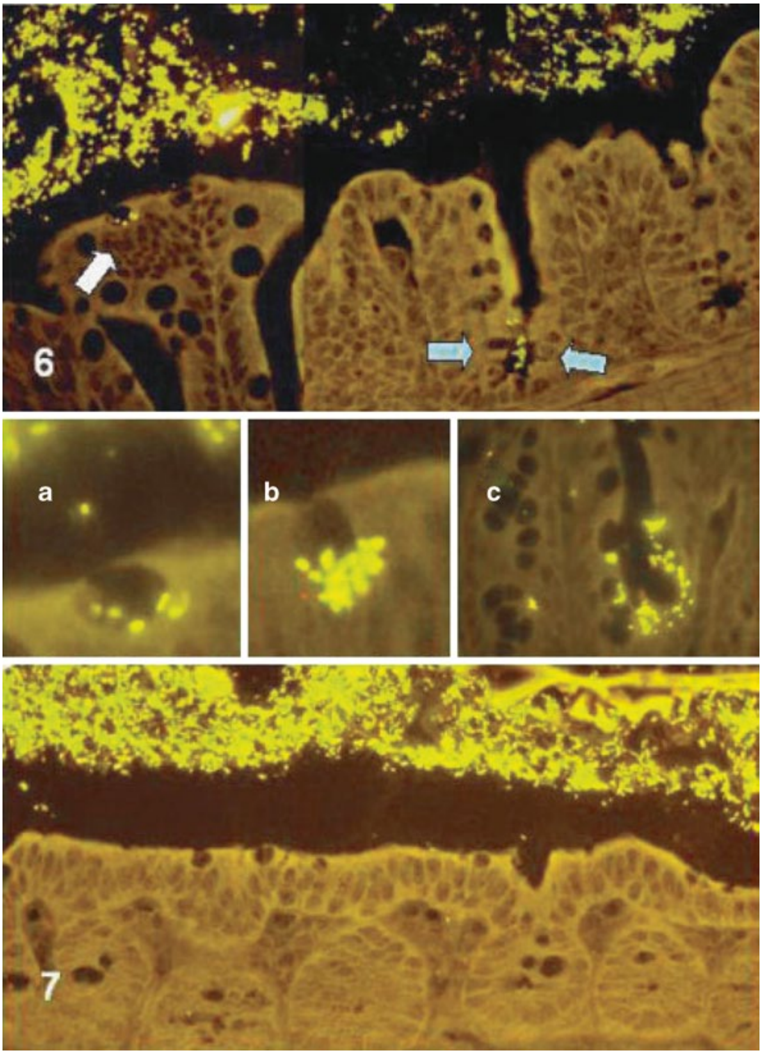


Fig. 10.9 Distribution of *Enterobacter cloacae (yellow fluorescence)* within the proximal *(top)* and distal *(bottom)* colon of the mouse. Bacteria are clearly separated from the colonic wall by a mucus layer. In the proximal colon, bacteria can be found in some of the emptying vacuoles of goblet cells *(white arrow)* and within crypts *(blue arrows)*, sometimes infiltrating the epithelial cells of the crypt base. (Swidsinski et al. 2007b)

of workers have reported that fine particulate material becomes enveloped in mucin and transported distally. Therefore, microbiota that are specifically bound to mucin components of this layer, or whose passage through it is sufficiently delayed, are likely to transit with the mucin or to be shed into the lumen and transit the intestine with other non-adherent particulate matter (Tirosh and Rubinstein 1998).

10.4.4.3 The Morphology of the Commensal Biofilm

Bacteria in the gastrointestinal tract may be free living in the liquid phase, or they may be attached either to the mucus layer of the intestinal mucosa or to solid particles within digesta where they form a biofilm (Macfarlane et al. 1999). A biofilm is an assemblage of microbes, often of different species, that is bound to a surface and with the component organisms sometimes enclosed in a matrix of secreted extracellular polysaccharides (EPS) (Donlan 2002). The properties of the mucin biofilm may contribute to the restrictions in mucin permeability, which were discussed earlier. Consequently, the thickness of the EPS within the biofilm community may be sufficient to impede the absorption of antibiotics (Donlan 2002). The architecture of the biofilm depends upon the component species and may influence the flow of fluid into the underlying surface. For instance, biofilms may on occasion contain interstitial voids that allow the passage of fluids and nutrients between constituent micro-colonies (Lewandowski 2000). The attachment of component species of microflora and the resultant architecture of the biofilm are influenced by flow, i.e. shear at the attachment surface. Moderate flow reduces the thickness of the boundary layer at the interface with a solid substrate and facilitates microbial attachment to it (Rijnaarts et al. 1993; Zheng et al. 1994). However, the structure of biofilms that are adherent to biological substrates may be eroded at high shear rates, e.g. around 300 s^{-1} (Ymele-Leki and Ross 2007), a level that could conceivably be achieved in the mucus biofilm in the distal colon during mass peristalsis, but which would be unlikely in the biofilms that are adherent to the digesta particles within faecal masses.

The component species in biofilms on the surfaces and interstices of digesta particles differ from those in the mucosa (Fig. 10.10) (Macfarlane and Macfarlane 2004) and may more closely resemble those in the foregut of ruminants (Walker et al. 2008), being adapted to colonise and degrade cellulosic substrates (Flint et al. 2007).

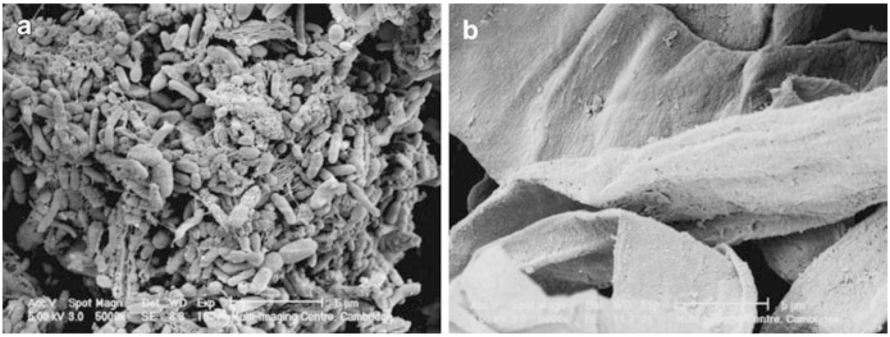


Fig. 10.10 SEM micrographs of food particles showing intestinal bacteria growing on the surface before washing (**a**) and after complete removal of adherent bacteria following treatment with a surfactant (**b**). (Macfarlane and Macfarlane 2006)

The relative proportions of the different bacterial species found in the liquid phase of digesta differ from those in the biofilms on digesta particles (Walker et al. 2008). In particular, *Ruminococcus* and *Rosburia* species are abundant in biofilms on particulate material and may be particularly adapted to bind with and intimately associate with solid substrates. Conversely, while *Bacteroides* species are able to degrade a variety of polysaccharides (Robert and Bernalier-Donadille 2003), they are less common in the biofilms on particulate digesta and comprise a higher proportion of the bacteria in the liquid phase (Tajima et al. 1999). Hence they may depend to a greater extent on the utilisation of solubilised polysaccharides, including those released by the activities of other bacteria (Walker et al. 2008).

In conclusion, it seems that the microbial digestion of suitable substrates in the colon can involve microbial species in three distinct loci and that the subsequent absorption of soluble products can require transit through up to two physically separate biofilms.

10.5 Microvilli

The boundary between the adherent mucus (glycocalyceal) layer and the enterocyte is morphologically complex, the apical plasma membrane being folded into a row of closely packed microvilli, i.e. the brush border (Fig. 10.11) (Horiuchi et al. 2005; Ito 1965). In human infants, there are around 40 microvilli of 100-nm diameter per 5 µm^2 of apical surface area and these are packed together with an inter-microvillous distance of around 25 nm (Phillips et al. 1979). Such dense packing restricts the para-microvillous spaces to molecular dimensions (Snoeck et al. 2005). Each microvillus comprises a vertical cylindrical extension of the enterocyte plasma membrane that is uniform in diameter and contains a polarised bundle of actin

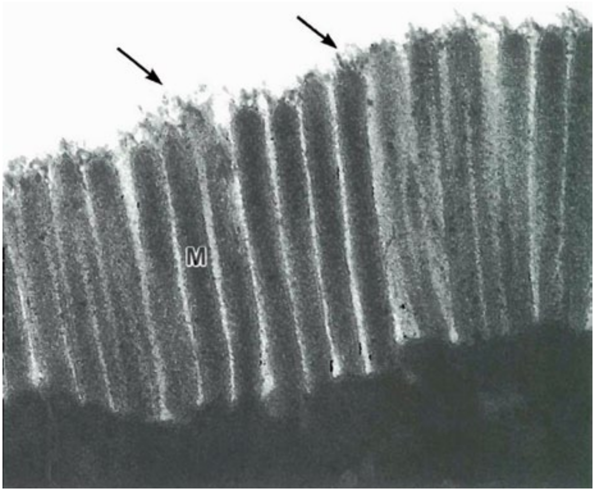


Fig. 10.11 TEM micrograph showing glycocalyces *(arrows)* adherent to brush border microvilli (M). (Horiuchi et al. 2005)

filaments linked to the overlying membrane by myosin motor proteins (Mooseker and Coleman 1989; Mooseker and Tilney 1975). These contractile elements appear to shorten and fan out when local cytosolic calcium ion concentrations increase (Mooseker 1985). It has long been held that microvilli provide a means of increasing apical absorptive surface area (Brown 1962). Moreover, modelling studies show that the close apposition of adjacent microvilli may enhance absorption rate by increasing surface area without increasing diffusion distance. Further, it has been shown that starvation increases the surface area of rat small intestinal microvilli along with amino acid transport capacity (Waheed and Gupta 1997).

Recent work has shown that microvilli regularly release vesicles of membrane-bound enzymes, including disaccharidases and alkaline phosphatases, into the adjacent (presumably submucus) intestinal lumen (McConnell et al. 2009). Such a mechanism fits with the finding that the apical plasma membrane is moved distally along the microvillus driven by myosin actin interactions (McConnell and Tyska 2007). Hence the increase of villous height in starved rats (Waheed and Gupta 1997) may reflect a reduction in the rate of release of vesicles and their consequent accumulation at the epithelial surface rather than a reactive increase in surface area.

These findings raise questions regarding the extent to which apical and subapical microvillous membranes act to augment absorption, and regarding the timing of the fracture of the intramembraneous SEA domains (Palmai-Pallag et al. 2005) of mucins on the apices of microvilli (Fig. 10.11). In regard to the former, it is evident that the closeness of apposition of adjacent microvilli and the net negative charge on the apical glycocalyx (Snoeck et al. 2005) form a significant barrier to the entry of viruses, bacteria and nanoparticles, in addition to that formed by the mucin layer. Further, given that the site of any absorptive invagination of the apical enterocytes' plasma membrane would be likely to be situated at the base of adjacent microvilli (Knutton et al. 1974), it is possible that the microvillous structure forms a significant barrier to the endocytosis of nutrient macromolecules.

With regard to the time of fracture of the apical glycocalyx, it is hypothesised that these structures will be preserved when the enterocytes bearing them are situated near to the bases of the intervillous spaces, but that they will be increasingly exposed to shear forces and be more likely to be fractured as the enterocytes migrate towards the tip of the villus. Electron microscopic studies show that the branched mucin glycoconjugates (Strous and Dekker 1992) that form the filamentous brush border glycocalyx (Ito 1965, 1974) are each anchored at a restricted microdomain at the microvillus tip that is small in comparison to the size of the structure (Maury et al. 1995). Moreover, they are readily separated from the mucosa by mechanical means (Ugolev et al. 1979).

It has long been known that the protein structures of the enzymes that are associated with the brush border contain sequences of amino acids with hydrophobic side groups, which associate with the lipids of the plasma membrane (Semenza 1986). Hence it is likely that such membrane binding will continue in enzymes such as maltose-glucoamylase, lactase-phlorizin hydrolase, sucrose isomaltase and dipeptidyl peptidase IV (McConnell et al. 2009), which are known to be associated with membranous vesicles following their release from microvilli.

If the vesicles and their associated enzymes are too large to transit the mucus layer, this would cause their activity to be confined to the aqueous phase adjacent to the enterocyte apex. Such confinement would effectively form a subsidiary compartment adjacent to the mucosa where nutrient oligomers would be cleaved into monomers prior to their absorption/endocytosis. A similar role has been suggested for the glycoclayx in the entrapment of pancreatic enzymes (Ugolev 1962; Ugolev and De Laey 1973). It has also been suggested the glycocalx could localise the ability of intramembraneous alkaline phosphatise to detoxify bacterial lipopolysaccharides in situ (Bates et al. 2007; Beumer et al. 2003; Goldberg et al. 2008; Su et al. 2006; van Veen et al. 2005). This, in conjunction with the general exclusion of bacteria from the submucus space by mucins, would serve to prevent nutrient monomers from being utilised by microbiota prior to their absorption.

The intestinal epithelium contains a number of islands of M cells that differ morphologically from enterocytes and are thought to be specialised in the sampling of antigens (Neutra et al. 1996). The bulk of these cells are found in Peyer's patches in the wall of the large and small intestine, and are associated with high concentrations of immune cells in the underlying submucosa. The thickness of the adherent layer of mucin overlying apical regions of the M cells is reduced to that of the glycocalyx, i.e. 500 nm (Frey et al. 1996). The microvilli in these regions are less densely packed, and there are broad areas of apical membrane through which endocytosis may occur (Frey et al. 1996). This morphology is thought to facilitate the endocytosis and vesicular transport of antigens to the associated immune cells. Experiments have examined the ability of subunit B of the cholera toxin, which was coupled to probes of various sizes, to bind to the membranes of M cells. These experiments showed that particles of 6.4 nm in diameter were able to bind to the apical membranes of all epithelial cells and M cells, particles of 28.8 nm failed to bind to enterocytes but were able to bind to M cells, while particles of 1.13 μm failed to bind either to enterocytes or to M cells. However, the latter particles did adhere to Caco-2 cells that lacked microvilli, but the particles failed to adhere to Caco-2 cells that developed microvilli (Frey et al. 1996). Together these findings show that the microvilli and the adherent glycocalyx of M cells may sterically hinder the approach of particles to the binding sites on the apical plasma membrane and the process of endocytosis.

10.6 Physiological Aspects of Absorption at the Mucosa

The enterocytes and the tight junctions, which link them, comprise the ultimate sites of nutrient absorption. Nutrients and ions can be absorbed or secreted either by apical or paracellular pathways.

10.6.1 Apical Transport

Apical transport of soluble substances can occur via intramembraneous pores. A series of these pores situated in the apical or apicolateral membrane may allow glucose and water to be transported from the lumen to the cytosol, and a series of pores in the basal or basolateral membrane may allow transport from the cytosol to the interstitial fluid of the subepithelial tissue. Transport across either of these membranes may be active or passive. Active systems generally involve specific transporter proteins, which can operate against diffusion and osmotic gradients, and expend energy in the form of ATP. Passive systems that allow the passage of hydrophilic moieties are simple hollow proteinous structures inserted in the plasma membrane, movement through the cavity being dependent upon concentration or osmotic gradients. Passive absorption of hydrophobic moieties may occur by their direct solution in the hydrophobic structure of the apical membrane. Transcellular transport can also occur via the binding of material to specific regions in the apical membrane followed by local invagination and internalisation of the contents. The transport of hydrophobic substances via this route may involve solution in the membrane leading to local changes in surface energy, which may engender invagination.

10.6.1.1 Membrane Transporters

Low molecular weight nutrients, such as sugars, amino acids and ions, may be absorbed by specific transporters located in the apical membranes of enterocytes. Transcellular transport of such nutrients is an active process moving ions against an electrical or concentration gradient using energy from the hydrolysis of ATP (Ma and Anderson 2006). For instance, Na^+ K^+ ATPase moves three Na^+ ions out of the basolateral membrane of enterocytes (i.e. their lateral membranes below the point at which they closely associate to form a continuous ‘tight junction’) in exchange for two K^+ ions. The electrical and ionic gradients created by such active transport processes can be harnessed to secondarily transport nutrients and water against concentration gradients. Thus, in the apical membrane of resting small intestinal enterocytes, the inward Na^+ gradient is used to transport glucose and water from the lumen to the enterocyte, against the concentration gradient, by the sodium-coupled glucose transporter SGLT1 (Ma and Anderson 2006). In humans, this apical system transports two Na^+ ions and one glucose molecule along with 240 water molecules, the latter being transported in physical association with the transported non-aqueous substrates (Zeuthen 2010). It is noteworthy that the rate of production of SGLT1 is increased when glucose is detected in the submucosal space by intestinal taste receptors (Dyer et al. 2005). Apical glucose and water absorption are also augmented by translocated GLUT2 after the consumption of a meal (Kellett 2001), whereby the ingress of one glucose molecule is accompanied by 30–40 water molecules that are in physical association with it (Zeuthen et al. 2007). Similar co-transport via K-Cl co-transport pump (KCC) systems situated in the basal membrane of small intestinal enterocytes is responsible for the transit of absorbed glucose from the enterocyte to the subepithelial interstitial space, each molecule being accompanied by 500 molecules of water. Together, these systems account for the bulk of absorptive water transit through the enterocyte, a process that can proceed in the face of adverse osmotic gradients from lumen to enterocyte cytosol, which can be 250 mOsm higher than that from blood plasma to tissue after a meal (Pappenheimer 1998).

As detailed earlier, the absorption of water via osmotic gradients can be augmented under certain circumstances by increasing the numbers of passive pores, such as those provided by aquaporins (Zeuthen 2010). However, the overall density of aquaporins is low in intestinal epithelium compared with that in other tissues, presumably to facilitate 'uphill' absorption against an osmotic gradient (Zeuthen 2010). As a result, it is likely that there is relatively little transmucosal flux of water and accompanying solutes from osmosis at the apices of enterocytes compared with that resulting from the operation of co-transporter and uniporter systems. It is noteworthy that the latter systems can allow the passive transport of small hydrophilic molecules, these moieties moving within the water space surrounding the actively absorbed substances. For example, urea may be passively co-transported by SGLT1 (Leung et al. 2000; MacAulay et al. 2002), a situation that could be considered to be broadly analogous to solvent drag. Of course, these considerations do not apply to the movement of water through the mucin layer, and hence, regardless of the means by which water is absorbed at the apical surfaces of enterocytes, there will be a compensatory influx of liquid through the mucin layer along with contained solutes to replace that which has been absorbed.

Less is understood regarding the role of membrane transport systems in the absorption of larger molecules, such as long-chain fatty acids. In the case of the absorption of medium- and long-chain fatty acids, while a number of transporting and binding proteins are associated with the apical membranes of enterocytes, it is currently unclear whether these function as true transmembrane transporters (Hansen et al. 2007, 2009) (see Chap. 5).

10.6.1.2 Endocytosis

Macromolecules can enter the apices of enterocytes via two distinct types of endocytosis, clathrin-mediated endocytosis and calveolae-mediated endocytosis. Clathrin-mediated endocytosis constitutes the major pathway of endocytosis and comprises a specific receptor-mediated absorption in short-lived pits formed on the plasma membrane (Gonnella and Neutra 1984; Stern and Walker 1984). Calveolae-mediated endocytosis occurs via flask-shaped invaginations of cholesterol- and sphingolipid-rich plasma membrane. Calveolae function to absorb a variety of molecules that are ligand-bound and conveyed either to the basement membrane or to cellular organelles by a number of alternative intracellular pathways.

In the case of fat absorption, recent electron micrographic evidence suggests that scavenger receptors (SR-B1) (Graf et al. 1999) some of which are located within the plasma membrane of caveolae in the apices of microvilli and in the areas between the bases of spaces between adjacent microvilli (Hansen et al. 2007, 2009) (Fig. 10.12) may act as docking sites for fats. These sites may allow direct uptake via a non aqueous pathway (Rodrigueza et al. 1999) or may act simply as a binding site rather than a simple conduit (Silver et al. 2001) perhaps by partitioning of their lipophilic elements into the fat droplet (Hansen et al. 2003). In the latter case endocytosis may be achieved by a flip-flop transmucosal transfer system (Stahl 2004) mediated by changes in the surface energy of the plasma membrane.

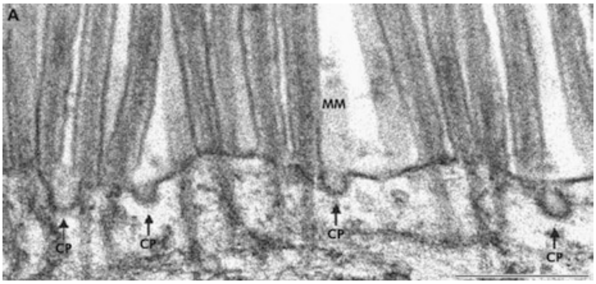


Fig. 10.12 Electron micrograph of the brush border region of an enterocyte showing many coated pits (CP) between adjacent microvilli (MM). The CP facilitate the endocytosis of fats. (Hansen et al. 2003)

Macromolecules that are taken up via the two types of endocytosis may subsequently be degraded by lysosomes or may avoid degradation. The latter may transit directly to the interstitial space or be presented to elements of the immune system (e.g. T cells) (Snoeck et al. 2005). In the case of fat absorption, further lipolysis of the contents is thought to be mediated by the co-endocytosis of intestinal alkaline phosphatase, a brush border vesicle enzyme (Hansen et al. 2007).

10.6.2 Paracellular Absorption

The membranes of adjacent enterocytes closely associate in the upper regions of their lateral surfaces to form encircling 'junction complexes'. Each complex is contiguous with those of adjacent cells forming a composite barrier, which spans the mucosa (Ballard et al. 1995). Within each junction complex, there are a series of subsidiary structures: 'tight junctions', which lie close to the apical end of the lateral aspect of the enterocyte; adherens junctions; and desmosomes, which lie deeper (Ma and Anderson 2006).

The tight junctions comprise an array of proteins that bridge the gap between the plasma membranes of adjacent cells, these include claudins (Furuse et al. 1998), occludins (Furuse et al. 1993) and other 'scaffolding proteins', such as zonulin (Fanning et al. 1998). Elements of the actin intracellular cytoskeletal system associate with these proteins (Barrios-Rodiles et al. 2005) and participate in the regulation of the tight junction integrity (Fanning et al. 1998; Madara and Carlson 1991).

The overall permeability of the epithelium is determined by the integrity of the tight junctions and is evidenced by its electrical resistance, and the size and charge of the molecules that are able to traverse it. The tight junction 'pore' has been reported to have a radius of 5 nm when open, which would allow the passage of macromolecules from 4–5.5 kDa (Pappenheimer 2001a; Pappenheimer et al. 1994). However, the pores have recently been reported to vary in size between 0.7–1.5 nm (7–15 Å) (Firth 2002; Salas and Moreno 1982; Watson et al. 2001), and a given tissue may contain a range of different tight junction pore sizes. Small intestinal mucosa and monolayers of (intestinal) Caco-2 cells are reported to be permeable to larger molecular probes, such as inulin (5,000 g/mol molecular wt), but to be more permeable to mannitol (343 g/mol molecular wt) by 2–3 orders of magnitude

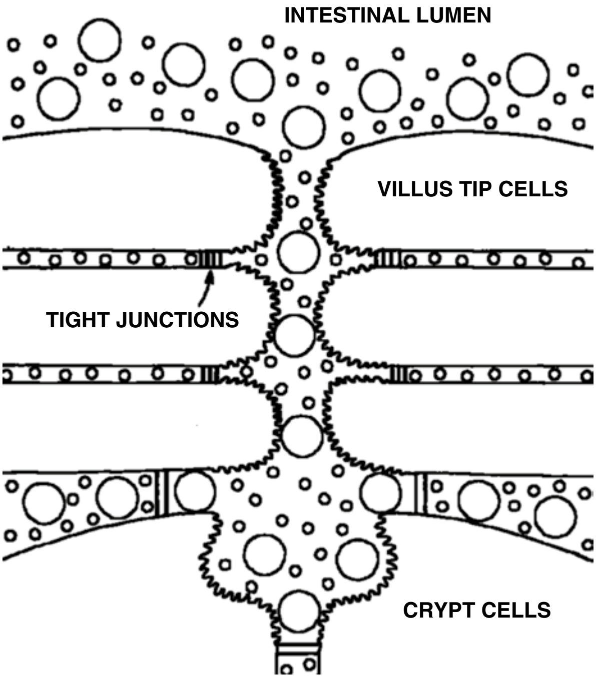


Fig. 10.13 Stylised model of the permeation of molecules through tight junctions of various sizes where tightness and permeability increase progressively from the crypt to the villus tip. (Bjarnason et al. 1995)

(Ma et al. 2000, 1991). The permeability of tight junctions also varies with the site of the mucosa in the gastrointestinal tract. The tight junctions in the mucosa of the gallbladder and small intestine mucosa are classified as 'leaky', while those in the colonic and gastric antral mucosa are classified as 'moderately tight', and those in the gastric fundal and oesophageal mucosa are classified as 'tight' (Diamond 1978; Powell 1981). Morphological studies show the tightness of intercellular tight junctions also varies at particular sites, e.g. paracellular tightness tends to increase from the base of the crypt to the tip of the villus within the small intestinal mucosa (Fig. 10.13) (Gumbiner 1987; Marcial et al. 1984).

In vivo tests of intestinal permeability similarly indicate that there may be a range of pore sizes distributed across the depth of the villous mucosa. For instance, the permeation of lactulose may be limited to the larger pores within the crypt junction whereas monosaccharides and polyethylene glycol 400 probes are able to permeate pores along the entire villous axis (Bjarnason et al. 1995). It is interesting to note that synchronous absorption of the large and the small probes would indicate that significant mixing occurred in the intervillous spaces.

10.6.3 Absorption: Passive Flux and Solvent Drag

In sites where the tight junctions are classified as leaky (Diamond 1978; Powell 1981), such as the small intestine, the paracellular pathway may provide a significant route for the transit of water and of ions by passive flux (Ma et al. 1991; Pappenheimer and Reiss 1987). In passive flux, the direction of transit is regulated by the osmotic gradient and the hydrostatic pressure across the tight junction. Thus as the osmolarity of material within the gut lumen decreases, the flux of water from the apicolateral intercellular space to the basolateral lateral space increases (Ma et al. 1991). Similarly, an increase in the osmolarity of the perfusate on the mucosal side of guinea pig jejunal mucosa mounted in an Ussing chamber causes the volume of the paracellular spaces to decrease, and both the transepithelial resistance (TER) and number of tight junction strands to increase (Madara 1983). Conversely, perfusion of a similarly 'leaky' preparation of gall bladder mucosa with a solution of low osmolarity causes the intercellular spaces to increase and the tight junctions to separate (Loeschke and Bentzel 1994).

It is important to note that water transiting the paracellular route can also carry solutes and ions with it, by solvent drag (Ma et al. 1991; Mullen et al. 1985; Pappenheimer and Reiss 1987). As a result, the passive transport of markers through tight junctions is governed by a combination of simple molecular diffusion and solvent drag, i.e. convective movements due to the bulk movement of solvent. The total flux (i.e. mass flow rate per unit area) of a solute (J_s) may be calculated:

$$J_s = J_D + J_{sd}$$

where J_D is the diffusive flux given by Fick's law and J_{sd} is the solute flux by solvent drag, which is given by:

$$J_{sd} = J_v \left(1 - \sigma_f\right) \bar{c}$$

where J_v is the solvent velocity (i.e. volumetric flow per unit area), $\bar{c}$ is the mean solute concentrations in the pore and σ_f is the solvent drag reflection coefficient indicating the proportion of the solute that is reflected back from the pore.

Studies indicate that the diffusive component of J_s is relatively small for a variety of marker solutes (Pappenheimer and Reiss 1987). However, it is important to note that the routine for the preparation of the intestinal segment that was used by these workers was unlikely to have removed the adherent mucus layer and may not have removed the mobile mucin layer. Consequently, their σ_f values may include an effect from these layers. Similarly, while it is known that claudin proteins are able to create charge selective pores at tight junctions (Ma and Anderson 2006), these effects may be confounded in intact preparations of intestinal epithelia by the charge selectivity of mucins.

The size of the tight junction, and hence the volume of passive flux, is also regulated by the amount of absorption of particular solutes that is occurring at the apex of the enterocyte. This phenomenon was identified indirectly from the observation that the absorption of glucose continues to increase when lumen concentrations exceed 10–15 mM, the point at which the active transport of glucose via transcellular absorption becomes saturated (Pappenheimer and Reiss 1987) This led to the hypothesis that activation of Na^+-glucose co-transport increases paracellular permeability, and that solvent drag from the increased water flux was responsible for the increase in absorption when higher concentrations of glucose were being perfused (Pappenheimer and Reiss 1987). A similar mechanism was proposed to operate during the absorption of amino acids (Pappenheimer and Reiss 1987). Subsequent work on hamster intestine confirmed that the addition of glucose or aminoacids to perfusates brought about a twofold or threefold decrease in junctional resistance (Pappenheimer and Reiss 1987). Work measuring the transit of non-absorbable markers, such as ferrocyanide, indicated that solvent drag may account for up to 90% of the glucose that is absorbed following the consumption of a meal (Pappenheimer 1988, 1990). Other work showed that it is the interaction of SGLT1 (Turner et al. 1997) with the sodium hydrogen exchanger (NHE) that activates the myosin light-chain kinase, and hence mediates the opening of the tight junction.

At the time of writing, the structural modulations of the components of tight junctions in response to Na^+-glucose co-transport, and those in response to pro-inflammatory cytokines and the presence of bacterial pathogens, remain incompletely understood (Shen and Turner 2006). It is also noteworthy that the validity of models that postulate absorption of significant volumes of water as a result of higher osmolarity in the lateral intercellular spaces have recently been challenged (Zeuthen 2010). More specifically, the permeabilities of epithelial cell membranes are generally not high (Hill 2008; Zeuthen et al. 1996) and detailed in vitro studies with microelectrodes placed in intact mucosa have shown that the concentration of the solution in intact lateral intercellular spaces does not differ significantly from that of the solution in which the tissue is bathed (Ikonomov et al. 1985; Zeuthen 1983).

10.6.4 Secretion: Concerted Action Between Apical and Passive Paracellular Routes

Leaky tight junctions, in conjunction with transcellular movements of ions, are able to mobilise large quantities of fluid into the intestinal lumen. Hence, when chloride channels in the enterocyte apex are opened, chloride ions transit down the electrochemical gradient. This creates a net local charge that induces electrochemical movement of sodium ions from the serosal to the luminal side of the tight junctions. This in turn generates an osmotic gradient causing water to transit the tight junction from the serosa to the lumen. This mechanism may account for much of the secretory movement of water in the proximal intestine (Ma and Anderson 2006).

References

Akiba Y, Guth PH, Engel E, Nastaskin I, Kaunitz JD (2000) Dynamic regulation of mucus gel thickness in rat duodenum. Am J Physiol 279:G437-447

Allen A (1978) Structure of gastrointestinal mucus glycoproteins and the viscous and gel-forming properties of mucus. Br Med Bull 34:28-33

Allen A, Flemström G (2005) Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. Am J Physiol 288:G1-19

Amerongen HM, Weltzin R, Farnet CM, Michetti P, Haseltine WA, Neutra MR (1991) Transepithelial transport of HIV-1 by intestinal M cells: a mechanism for transmission of AIDS. J Acquir Immune Defic Syndr 4:760-765

Amidon GL, Kou J, Elliott RL, Lightfoot EN (1980) Analysis of models for determining intestinal wall permeabilities. J Pharm Sci 69:1369-1373

Amsden B (1998) Solute diffusion within hydrogels. Mechanisms and models. Macromolecules 31:8382-8395

Amsden B (1999) An obstruction-scaling model for diffusion in homogeneous hydrogels. Macromolecules 32:874-879

Amsden B, Turner N (1999) Diffusion characteristics of calcium alginate gels. Biotechnol Bioeng 65:605-610

Andrews G, Laverty T, Jones D (2009) Mucoadhesive polymeric platforms for controlled drug delivery. Eur J Pharm Biopharm 71:505-518

Assentoft JE, Gregersen H, O'Brien WD (2000) Determination of Biomechanical Properties in Guinea Pig Esophagus by Means of High Frequency Ultrasound and Impedance Planimetry. Dig Dis Sci 45:1260-1266

Atuma C, Strugala V, Allen A, Holm L (2001) The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. Am J Physiol 280:G922-929

Audie JP, Janin A, Porchet N, Copin MC, Gosselin B, Aubert JP (1993) Expression of human mucin genes in respiratory, digestive, and reproductive tracts ascertained by *in situ* hybridization. J Histochem Cytochem 41:1479-1485

Axelsson MAB, Asker N, Hansson GC (1998) O-glycosylated MUC2 monomer and dimer from LS 174T cells are water-soluble, whereas larger MUC2 species formed early during biosynthesis are insoluble and contain nonreducible intermolecular bonds. J Biol Chem 273:18864-18870

Ballard S, Hunter J, Taylor A (1995) Regulation of tight-junction permeability during nutrient absorption across the intestinal epithelium. Annu Rev Nutr 15:35-55

Barker N, Van De Wetering M, Clevers H (2008) The intestinal stem cell. Genes Dev 22:1856-1864

Barrios-Rodiles M, Brown KR, Ozdamar B, Bose R, Liu Z, Donovan RS, Shinjo F, Liu Y, Dembowy J, Taylor IW (2005) High-throughput mapping of a dynamic signaling network in mammalian cells. Science 307:1621-1625

Barry Jr R (1976) Mucosal surface areas and villous morphology of the small intestine of small mammals: functional interpretations. J Mammal 57:273-290

Bates JM, Akerlund J, Mittge E, Guillemin K (2007) Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. Cell Host Microbe 2:371-382

Bell L, Williams L (1982) A scanning and transmission electron microscopical study of the morphogenesis of human colonic villi. Anat Embryol (Berl) 165:437-455

Beumer C, Wulferink M, Raaben W, Fiechter D, Brands R, Seinen W (2003) Calf intestinal alkaline phosphatase, a novel therapeutic drug for lipopolysaccharide (LPS)-mediated diseases, attenuates LPS toxicity in mice and piglets. J Pharmacol Exp Ther 307:737-744

Bhaskar KR, Garik P, Turner BS, Bradley JD, Bansil R, Stanley HE, LaMont JT (1992) Viscous fingering of HCl through gastric mucin. Nature 360:458-461

Bijlsma PB, Peeters RA, Groot JA, Dekker PR, Taminiau J, A,J.M, van der Meer R (1995) Differential *in vivo* and *in vitro* intestinal permeability to lactulose and mannitol in animals and humans: a hypothesis. Gastroenterology 108:687-696

Bjarnason I, Macpherson A, Hollander D (1995) Intestinal permeability: an overview. Gastroenterology 108:1566-1581

Bond JH, Levitt DG, Levitt MD (1977) Quantitation of countercurrent exchange during passive absorption from the dog small intestine: evidence for marked species differences in the efficiency of exchange. J Clin Invest 59:308-318

Bongaerts G, Severijnen R, Timmerman H (2005) Effect of antibiotics, prebiotics and probiotics in treatment for hepatic encephalopathy. Med Hypotheses 64:64-68

Bongaerts JHH, Rossetti D, Stokes JR (2007) The lubricating properties of human whole saliva. Trib Lett 27:277-287

Boshi Y, Nezu R, Khan J (1996) Developmental changes in distribution of the mucous gel layer and intestinal permeability in rat small intestine. J Parenter Enteral Nutr 20:406-411

Brown AL (1962) Microvilli of the human jejunal epithelial cell. J Cell Biol 12:623-627

Bry L, Falk PG, Midtvedt T, Gordon JI (1996) A model of host-microbial interactions in an open mammalian ecosystem. Science 273:1380-1383

Bustamante SA, Jodal M, Nilsson NJ, Lundgren O, Köhlin T (1989) Evidence for a countercurrent exchanger in the intestinal villi of suckling swine. Acta Physiol Scand 137:207-213

Cammarota G, Martino A, Pirozzi GA, Cianci R, Cremonini F, Zuccalà G, Cuoco L, Ojetti V, Montalto M, Vecchio FM (2004) Direct visualization of intestinal villi by high-resolution magnifying upper endoscopy: a validation study. Gastrointest Endosc 60:732-738

Cao X, Bansil R, Bhaskar K, Turner B, LaMont J, Niu N, Afdhal N (1999) pH-dependent conformational change of gastric mucin leads to sol-gel transition. Biophys J 76:1250-1258

Carlstedt I, Sheehan JK (1984) Macromolecular properties and polymeric structure of mucus glycoproteins. Mucus and Mucosa, Ciba Found Symp. Pitman, pp 157-172

Carrión F, Martínez-Nicolás G, Iglesias P, Sanes J, Bermúdez MD (2009) Liquid Crystals in Tribology. Int J Mol Sci 10:4102-4115

Celli J, Gregor B, Turner B, Afdhal NH, Bansil R, Erramilli S (2005) Viscoelastic properties and dynamics of porcine gastric mucin. Biomacromolecules 6:1329-1333

Celli JP, Turner BS, Afdhal NH, Ewoldt RH, McKinley GH, Bansil R, Erramilli S (2007) Rheology of Gastric Mucin Exhibits a pH-Dependent Sol-Gel Transition. Biomacromolecules 8:1580-1586

Chambraud L, Bernadac A, Gorvel JP, Maroux S (1989) Renewal of goblet cell mucus granules during the cell migration along the crypt-villus axis in rabbit jejunum: an immunolabeling study. Biol Cell 65:159-162

Chang EB, Rao MC (1994) Intestinal water and electrolyte transport: mechanisms of physiological and adaptive responses. In: Johnson LR (ed) Physiology of the Gastrointestinal Tract. Raven, New York, pp 2027-2081

Chang SK, Dohrman AF, Basbaum CB, Ho SB, Tsuda T, Toribara NW, Gum JR, Kim YS (1994) Localization of mucin (MUC2 and MUC3) messenger RNA and peptide expression in human normal intestine and colon cancer. Gastroenterology 107:28-36

Cone RA (1999) Mucus. In: Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee JR (eds) Mucosal immunology. Academic Press, San Diego, pp 43-64

Cone RA (2009) Barrier properties of mucus. Adv Drug Deliv Rev 61:75-85

Copeman M, Matuz J, Leonard AJ, Pearson JP, Dettmar PW, Allen A (1994) The gastroduodenal mucus barrier and its role in protection against luminal pepsins: the effect of 16,16 dimethyl prostaglandin E2, carbopol-polyacrylate, sucralfate and bismuth subsalicylate. J Gastroenterol Hepatol 9:55-59

Corfield AP, Wiggins R, Edwards C, Myerscough N, Warren BF, Soothill P, Millar MR, Homer P (2002) A sweet coating - how bacteria deal with sugars. In: Axford JS (ed) Glycobiology and Medicine, pp 3-15

Corthesy B (2003) Recombinant secretory immunoglobulin A in passive immunotherapy: linking immunology and biotechnology. Curr Pharm Biotechnol 4:51-67

Creamer B (1964) Variations in small-intestinal villous shape and mucosal dynamics. Br Med J 2:1371-1373

Cu Y, Saltzman W (2009) Mathematical modeling of molecular diffusion through mucus. Adv Drug Deliv Rev 61:101-114

Davies JM, Viney C (1998) Water–mucin phases: conditions for mucus liquid crystallinity. Thermochimica Acta 315:39-49

Dawson M, Wirtz D, Hanes J (2003) Enhanced Viscoelasticity of Human Cystic Fibrotic Sputum Correlates with Increasing Microheterogeneity in Particle Transport. J Biol Chem 278:50393-50401

De Bolós C, Garrido M, Real FX (1995) MUC6 apomucin shows a distinct normal tissue distribution that correlates with Lewis antigen expression in the human stomach. Gastroenterology 109:723-734

De Santa Barbara P, Van Den Brink GR, Roberts DJ (2003) Development and differentiation of the intestinal epithelium. Cell Mol Life Sci 60:1322-1332

Debnam ES, Levin RJ (1975) Effects of fasting and semistarvation on the kinetics of active and passive sugar absorption across the small intestine in vivo. J Physiol 252:681-700

Deplancke B, Gaskins HR (2001) Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J Clin Nutr 73:1131S-1141S

Desai MA, Mutlu M, Vadgama P (1992) A study of macromolecular diffusion through native porcine mucus. Cell Mol Life Sci 48:22-26

DeSimone JA (1983) Diffusion barrier in the small intestine. Science 220:221-222

Diamond JM (1978) Channels in epithelial cell membranes and junctions. Fed Proc 37:2639-2643

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8:881-890

Dou Y, Gregersen S, Zhao J, Zhuang F, Gregersen H (2002) Morphometric and biomechanical intestinal remodeling induced by fasting in rats. Dig Dis Sci 47:1158-1168

Dresselhuis DM, De Hoog EHA, Cohen Stuart MA, Van Aken GA (2008) Application of oral tissue in tribological measurements in an emulsion perception context. Food Hydrocolloids 22:323-335

Dyer J, Salmon K, Zibrik L, Shirazi-Beechey S (2005) Expression of sweet taste receptors of the T1R family in the intestinal tract and enteroendocrine cells. Biochem Soc Trans 33:302-305

Enss ML, Müller H, Schmidt-Wittig U, Kownatzki R, Coenen M, Hedrich HJ (1996) Effects of perorally applied endotoxin on colonic mucins of germfree rats. Scand J Gastroenterol 31:868-874

Fagerholm U, Lennernäs H (1995) Experimental estimation of the effective unstirred water layer thickness in the human jejunum, and its importance in oral drug absorption. Eur J Pharm Sci 3:247-253

Fanning AS, Jameson BJ, Jesaitis LA, Anderson JM (1998) The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. J Biol Chem 273:29745-29753

Fettiplace R, Haydon DA (1980) Water permeability of lipid membranes. Physiol Rev 60:510-550

Fine KD, Santa Ana CA, Porter JL, Fordtran JS (1995) Effect of changing intestinal flow rate on a measurement of intestinal permeability. Gastroenterology 108:983-989

Firth JA (2002) Endothelial barriers: From hypothetical pores to membrane proteins. J Anat 200:524-525

Flint HJ, Duncan SH, Scott KP, Louis P (2007) Interactions and competition within the microbial community of the human colon: links between diet and health. Environ Microbiol 9:1101-1111

Florey HW (1962) The secretion and function of intestinal mucus. Gastroenterology 43:326-329

Frey A, Giannasca KT, Weltzin R, Giannasca PJ, Reggio H, Lencer WI, Neutra MR (1996) Role of the glycocalyx in regulating access of microparticles to apical plasma membranes of intestinal epithelial cells: implications for microbial attachment and oral vaccine targeting. J Exp Med 184:1045-1059

Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S (1998) Claudin-1 and-2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 141:1539-1550

Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S (1993) Occludin: a novel integral membrane protein localizing at tight junctions. J Cell Biol 123:1777-1788

Goldberg R, Austen WG, Zhang X, Munene G, Mostafa G, Biswas S, McCormack M, Eberlin KR, Nguyen JT, Tatlidede HS (2008) Intestinal alkaline phosphatase is a gut mucosal defense factor maintained by enteral nutrition. Proc Natl Acad Sci USA 105:3551-3556

Gonnella PA, Neutra MR (1984) Membrane-bound and fluid-phase macromolecules enter separate prelysosomal compartments in absorptive cells of suckling rat ileum. J Cell Biol 99:909-917

Graf G, Matveev S, Smart E (1999) Class B scavenger receptors, caveolae and cholesterol homeostasis. Trends Cardiovasc Med 9:221-224

Greenberg EP, Canale-Parola E (1977) Motility of flagellated bacteria in viscous environments. J Bacteriol 132:356-358

Gregersen H (2003) Biomechanics of the gastrointestinal tract: new perspectives in motility research and diagnostics. Springer Verlag, New York

Gregersen H, Kassab G (1996) Biomechanics of the gastrointestinal tract. Neurogastroenterol Mot 8:277-297

Gregersen H, Kassab G, Pallencaoe E, Lee C, Chien S, Skalak R, Fung Y (1997) Morphometry and strain distribution in guinea pig duodenum with reference to the zero-stress state. American Journal of Physiology- Gastrointestinal and Liver Physiology 273:865

Gruber P, Longer MA, Robinson JR (1987) Some biological issues in oral, controlled drug delivery. Adv Drug Deliv Rev 1:1-18

Gruzdkov AA, Gusev V, Ugolev AM (1989) Mathematical modeling. In: Ugolev AM (ed) Membrane digestion: new facts and concepts. Mir, Moscow, pp 228–234

Güldner FH, Wolff JR, Keyserlingk DG (1972) Fibroblasts as a part of the contractile system in duodenal villi of rat. Cell Tissue Res 135:349-360

Gum JR, Hicks J, Toribara NW, Siddiki B, Kim Y (1994) Molecular cloning of human intestinal mucin (MUC2) cDNA. Identification of the amino terminus and overall sequence similarity to prepro-von Willebrand factor. J Biol Chem 269:2440-2446

Gumbiner B (1987) Structure, biochemistry, and assembly of epithelial tight junctions. Am J Physiol 253:C749-758

Haljamäe H, Jodal M, Lundgren O (1973) Countercurrent multiplication of sodium in intestinal villi during absorption of sodium chloride. Acta Physiol Scand 89:580-593

Hallbäck DA, Hulten L, Jodal M, Lindhagen J, Lundgren O (1978) Evidence for the existence of a countercurrent exchanger in the small intestine in man. Gastroenterology 74:683-690

Hallbäck DA, Jodal M, Mannischeff M, Lundgren O (1991) Tissue osmolality in intestinal villi of four mammals in vivo and in vitro. Acta Physiol Scand 143:271-277

Hansen GH, Niels-Christiansen LL, Immerdal L, Danielsen EM (2003) Scavenger receptor class B type I (SR-BI) in pig enterocytes: trafficking from the brush border to lipid droplets during fat absorption. Gut 52:1424-1431

Hansen GH, Niels-Christiansen LL, Immerdal L, Nystrom BT, Danielsen EM (2007) Intestinal alkaline phosphatase: selective endocytosis from the enterocyte brush border during fat absorption. Am J Physiol 293:G1325-1332

Hansen GH, Rasmussen K, Niels-Christiansen LL, Danielsen EM (2009) Endocytic trafficking from the small intestinal brush border probed with FM dye. Am J Physiol 297:G708-715

Harada N, Iijima S, Kobayashi K, Yoshida T, Brown WR, Hibi T, Oshima A, Morikawa M (1997) Human IgGFc binding protein (FcγBP) in colonic epithelial cells exhibits mucin-like structure. J Biol Chem 272:15232-15241

Harris MS, Kennedy JG (1988) Relationship between distention and absorption in rat intestine. II. Effects of volume and flow rate on transport. Gastroenterology 94:1172-1179

Hasan M, Ferguson A (1981) Measurements of intestinal villi non-specific and ulcer-associated duodenitis-correlation between area of microdissected villus and villus epithelial cell count. J Clin Pathol 34:1181-1186

Haward SJ, Odell JA, Berry M, Hall T (2010) Extensional rheology of human saliva. Rheol Acta DOI 10.1007/s00397-010-0494-1

Herrmann A, Davies JR, Lindell G, Mårtensson S, Packer NH, Swallow DM, Carlstedt I (1999) Studies on the "insoluble" glycoprotein complex from human colon. Identification of reduction-insensitive MUC2 oligomers and C-terminal cleavage. J Biol Chem 274:15828-15828

Hill AE (2008) Fluid transport: a guide for the perplexed. J Membr Biol 223:1-11

Ho SB, Anway RE, Ahmed Z (2000) Spatial organization of MUC5AC and MUC5 mucins within the surface mucous layer of the stomach. Gastroenterology 118:1396

Hollander D (1992) The intestinal permeability barrier: a hypothesis as to its regulation and involvement in Crohn's disease. Scand J Gastroenterol 27:721-726

Holm L, Flemström G (1990) Microscopy of acid transport at the gastric surface *in vivo*. J Intern Med 732:91-95

Holzheimer G, Winne D (1989) Influence of distension on absorption and villous structure in rat jejunum. Am J Physiol 256:G188-197

Hong Z, Chasan B, Bansil R, Turner B, Bhaskar K, Afdhal N (2005) Atomic force microscopy reveals aggregation of gastric mucin at low pH. Biomacromolecules 6:3458-3466

Hooper LV, Gordon JI (2001) Glycans as legislators of host-microbial interactions: spanning the spectrum from symbiosis to pathogenicity. Glycobiology 11:1R-10R

Hooper LV, Xu J, Falk PG, Midtvedt T, Gordon JI (1999) A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. Proc Natl Acad Sci USA 96:9833-9838

Horiuchi K, Naito I, Nakano K, Nakatani S, Nishida K, Taguchi T, Ohtsuka A (2005) Three-dimensional ultrastructure of the brush border glycocalyx in the mouse small intestine: a high resolution scanning electron microscopic study. Arch Histol Cytol 68:51-56

Hoskins LC, Boulding ET (1981) Mucin degradation in human colon ecosystems: evidence for the existence and role of bacterial subpopulations producing glycosidases as extracellular enzymes. J Clin Invest 67:163-172

Hosoyamada Y, Sakai T (2007) Mechanical components of rat intestinal villi as revealed by ultrastructural analysis with special reference to the axial smooth muscle cells in the villi. Arch Histol Cytol 70:107-116

Hume ID (1999) Marsupial Nutrition. Cambridge University Press, Cambridge

Ihida K, Suganuma T, Tsutama S, Murata F (1988) Glycoconjugate histochemistry of the rat fundic gland using *Griffonia simplicifolia* agglutinin-II during the development. Am J Anat 182:250-256

Ikonomov O, Simon M, Frömter E (1985) Electrophysiological studies on lateral intercellular spaces of *Necturus* gallbladder epithelium. Pflügers Arch 403:301-307

Isberg R, Van Nhieu G (1994) Two mammalian cell internalization strategies used by pathogenic bacteria. Annu Rev Genet 28:395-422

Ishihara K, Kurihara M, Eto H, Kasai K, Shimauchi S, Hotta K (1993) A monoclonal antibody against carbohydrate moiety of rat gastric surface epithelial cell-derived mucin. Hybridoma 12:609-620

Ito S (1965) The enteric surface coat on cat intestinal microvilli. J Cell Biol 27:475-491

Ito S (1974) Form and function of the glycocalyx on free cell surfaces. Philos Trans R Soc Lond B Biol Sci 268:55-66

Johansson M, Synnerstad I, Holm L (2000) Acid transport through channels in the mucous layer of rat stomach. Gastroenterology 119:1297-1304

Johansson MEV, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC (2008) The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci USA 105:15064-15069

Johansson MEV, Thomsson KA, Hansson GC (2009) Proteomic analyses of the two mucus layers of the colon barrier reveal that their main component, the Muc2 mucin, is strongly bound to the FcγBP protein. J Proteome Res 8:3549-3557

Johnson DA, Amidon GL (1988) Determination of intrinsic membrane transport parameters from perfused intestine experiments: a boundary layer approach to estimating the aqueous and unbiased membrane permeabilities. J Theor Biol 131:93-106

Junqueira LC, Carneiro J (2005) Basic Histology: Text & Atlas. McGraw-Hill, New York

Karlsson J, Artursson P (1991) A method for the determination of cellular permeability coefficients and aqueous boundary layer thickness in monolayers of intestinal epithelial (Caco-2) cells grown in permeable filter chambers. Int J Pharm 71:55-64

Karlsson NG, Herrmann A, Karlsson H, Johansson ME, Carlstedt I, Hansson GC (1997) The glycosylation of rat intestinal Muc2 mucin varies between rat strains and the small and large intestine. J Biol Chem 272:27025-27034

Karlsson NG, Johansson ME, Asker N, Karlsson H, Gendler SJ, Carlstedt I, Hansson GC (1996) Molecular characterization of the large heavily glycosylated domain glycopeptide from the rat small intestinal Muc2 mucin. Glycoconj J 13:823-831

Kas HS (1997) Chitosan: properties, preparations and application to microparticulate systems. J Microencapsul 14:689-711

Kellett GL (2001) The facilitated component of intestinal glucose absorption. J Physiol 531:585-595

Kerss S, Allen A, Garner A (1982) A simple method for measuring thickness of the mucus gel layer adherent to rat, frog and human gastric mucosa: influence of feeding, prostaglandin, N-acetylcysteine and other agents. Clin Sci 63:187-195

Khanvilkar K, Donovan MD, Flanagan DR (2001) Drug transfer through mucus. Adv Drug Deliv Rev 48:173-193

Kinter IS, Wilson TH (1975) Autoradiographic study of sugar and amino acid transport by everted sacs of hamster intestine. J Cell Biol 25:19-39

Kirjavainen PV, Ouwehand AC, Isolauri E, Salminen SJ (1998) The ability of probiotic bacteria to bind to human intestinal mucus. FEMS Microbiol Lett 167:185-189

Kleessen B, Blaut M (2007) Modulation of gut mucosal biofilms. Br J Nutr 93:35-40

Knutson T, Fridblom P, Ahlstrom H, Magnusson A, Tannergren C, Lennernas H (2009) Increased Understanding of Intestinal Drug Permeability Determined by the LOC-I-GUT Approach Using Multislice Computed Tomography. Mol Pharmaceut 6:2-10

Knutton S, Limbrick AR, Robertson JD (1974) Regular structures in membranes: I. Membranes in the Endocytic Complex of Ileal Epithelial Cells. J Cell Biol 62:679-694

Komuro T (1985) Fenestrations of the basal lamina of intestinal villi of the rat. Cell Tissue Res 239:183-188

Komuro T, Hashimoto Y (1990) Three-dimensional structure of the rat intestinal wall (mucosa and submucosa). Arch Histol Cytol 53:1-21

Lai S, Wang Y, Wirtz D, Hane0s J (2009) Micro-and macrorheology of mucus. Adv Drug Deliv Rev 61:86-100

Lee J (1969) A micropuncture study of water transport by dog jejunal villi in vitro. Am J Physiol 217:1528-1533

Lee S, Muller M, Rezwan K, Spencer ND (2005) Porcine gastric mucin (PGM) at the water/poly (dimethylsiloxane)(PDMS) interface: influence of pH and ionic strength on its conformation, adsorption, and aqueous lubrication properties. Langmuir 21:8344-8353

Lentle RG, Janssen PWM (2008) Physical characteristics of digesta and their influence on flow and mixing in the mammalian intestine: a review. J Comp Physiol B178:673-690

Leung DW, Loo DDF, Hirayama BA, Zeuthen T, Wright EM (2000) Urea transport by cotransporters. J Physiol 528:251-257

Levine MJ (1993) Salivary macromolecules. A structure/function synopsis. Ann N Y Acad Sci 694:11-16

Levitt MD, Fetzer CA, Kneip JM, Bond JH, Levitt DG (1987) Quantitative assessment of luminal stirring in the perfused small intestine of the rat. Am J Physiol 252:G325-332

Levitt MD, Furne JK, Strocchi A, Anderson BW, Levitt DG (1990) Physiological measurements of luminal stirring in the dog and human small bowel. J Clin Invest 86:1540-1547

Levitt MD, Kneip JM, Levitt DG (1988) Use of laminar flow and unstirred layer models to predict intestinal absorption in the rat. J Clin Invest 81:1365-1369

Lewandowski Z (2000) Structure and Function of Biofilms. In: Evans LV (ed) Biofilms: Recent advances in their study and control. Harwood Academic, Amsterdam, pp 1-17

Leyton L (1975) Fluid behaviour in biological systems. Oxford University Press

Lin JH, Chiba M, Baillie TA (1999) Is the role of the small intestine in first-pass metabolism overemphasized? Pharmacol Rev 51:135-157

Loeschke K, Bentzel CJ (1994) Osmotic water flow pathways across Necturus gallbladder: role of the tight junction. Am J Physiol 266:G722-730

Lu X, Gregersen H (2001) Regional distribution of axial strain and circumferential residual strain in the layered rabbit oesophagus. J Biomech 34:225-233

Ma TY, Anderson JM (2006) Tight junctions and the intestinal barrier. In: Johnson LR (ed) Physiology of the Gastrointestinal Tract. Academic Press, San Diego, pp 1559-1594

Ma TY, Hoa NT, Tran DD, Bui V, Pedram A, Mills S, Merryfield M (2000) Cytochalasin B modulation of Caco-2 tight junction barrier: role of myosin light chain kinase. Am J Physiol 279:G875-885

Ma TY, Hollander D, Erickson RA, Truong H, Krugliak P (1991) Is the small intestinal epithelium truly 'tight' to inulin permeation? Am J Physiol 260:G669-676

MacAulay N, Gether U, Klærke DA, Zeuthen T (2002) Passive water and urea permeability of a human Na^+–glutamate cotransporter expressed in *Xenopus* oocytes. J Physiol 542:817-828

Macfarlane S, Cummings JH, Macfarlane GT (1999) Bacterial colonisation of surfaces in the large intestine. In: Gibson GR, Roberfroid MB (eds) Colonic Microbiota, Nutrition and Health. Kluwer Academic, Dordrecht, pp 71–88

Macfarlane S, Macfarlane GT (2004) Bacterial diversity in the human gut. Adv Appl Microbiol 54:261-289

Macfarlane S, Macfarlane GT (2006) Composition and Metabolic Activities of Bacterial Biofilms Colonizing Food Residues in the Human Gut. Appl Environ Microbiol 72:6204-6211

Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA (1999) Probiotics inhibit enteropathogenic *E. coli* adherence *in vitro* by inducing intestinal mucin gene expression. Am J Physiol 276:G941-950

Madara JL (1983) Increases in guinea pig small intestinal transepithelial resistance induced by osmotic loads are accompanied by rapid alterations in absorptive-cell tight-junction structure. J Cell Biol 97:125-136

Madara JL, Carlson S (1991) Supraphysiologic L-tryptophan elicits cytoskeletal and macromolecular permeability alterations in hamster small intestinal epithelium *in vitro*. J Clin Invest 87:454-462

Mailman D, Womack WA, Kvietys PR, Granger DN (1990) Villous motility and unstirred water layers in canine intestine. Am J Physiol 258:G238-246

Marcial MA, Carlson SL, Madara JL (1984) Partitioning of paracellular conductance along the ileal crypt-villus axis: a hypothesis based on structural analysis with detailed consideration of tight junction structure-function relationships. J Membr Biol 80:59-70

Marshman E, Booth C, Potten CS (2002) The intestinal epithelial stem cell. Bioessays 24:91-98

Matsuo K, Ota H, Akamatsu T, Sugiyama A, Katsuyama T (1997) Histochemistry of the surface mucous gel layer of the human colon. Gut 40:782-789

Matthes I, Nimmerfall F, Vonderscher J, Sucker H (1992) Mucus models for investigation of intestinal absorption mechanisms. 4. Comparison of mucus models with absorption models *in vivo* and *in situ* for prediction of intestinal drug absorption. Pharmazie 47:787-791

Maury J, Nicoletti C, Guzzo-Chambraud L, Maroux S (1995) The filamentous brush border glycocalyx, a mucin-like marker of enterocyte hyper-polarization. Eur J Biochem 228:323-331

McConnell RE, Higginbotham JN, Shifrin Jr DA, Tabb DL, Coffey RJ, Tyska MJ (2009) The enterocyte microvillus is a vesicle-generating organelle. J Cell Biol 185:1285-1298

McConnell RE, Tyska MJ (2007) Myosin-1a powers the sliding of apical membrane along microvillar actin bundles. J Cell Biol 177:671-681

Menzies IS (1984) Transmucosal passage of inert molecules in health and disease. In: Skadhauge E, Heintze K (eds) Intestinal absorption and secretion (Falk Symposium 36). MTP Press, Titisee, West Germany, pp 527–543

Michel CC, Curry FE (1999) Microvascular permeability. Physiol Rev 79:703-761

Minekus M, Smeets-Peeters M, Bernalier A, Marol-Bonnin S, Havenaar R, Marteau P, Alric M, Fonty G, Huis In't Veld JHJ (1999) A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. Appl Microbiol Biotechnol 53:108-114

Mooseker M (1985) Organization, chemistry, and assembly of the cytoskeletal apparatus of the intestinal brush border. Annu Rev Cell Biol 1:209-241

Mooseker MS, Coleman TR (1989) The 110-kD protein-calmodulin complex of the intestinal microvillus (brush border myosin I) is a mechanoenzyme. J Cell Biol 108:2395-2400

Mooseker MS, Tilney LG (1975) Organization of an actin filament-membrane complex. Filament polarity and membrane attachment in the microvilli of intestinal epithelial cells. J Cell Biol 67:725-743

Mullen TL, Muller M, Van Bruggen JT (1985) Role of solute drag in intestinal transport. J Gen Physiol 85:347-363

Neutra M, Pringault E, Kraehenbuhl J (1996) Antigen sampling across epithelial barriers and induction of mucosal immune responses. Annu Rev Immunol 14:275-300

Neutra MR, Giannasca PJ, Giannasca KT, Kraehenbuhl JP (1995) M cells and microbial pathogens. In: Blaser MJ, Ravdin JI, Greenberg HB, Guerrant RL (eds) Infections of the GI tract. Raven, New York, pp 163–178

Neutra MR, Phillips TL, Mayer EL, Fishkind DJ (1987) Transport of membrane-bound macromolecules by M cells in follicle-associated epithelium of rabbit Peyer's patch. Cell Tissue Res 247:537-546

Nicholas CV, Desai M, Vadgama P, McDonnell MB, Lucas S (1991) pH dependence of hydrochloric acid diffusion through gastric mucus: correlation with diffusion through a water layer using a membrane-mounted glass pH electrode. Analyst 116:463-467

Niibuchi JJ, Aramaki Y, Tsuchiya S (1986) Binding of antibiotics to rat intestinal mucin. Int J Pharm 30:181-187

Nittmann J, Daccord G, Stanley HE (1985) Fractal growth of viscous fingers: quantitative characterization of a fluid instability phenomenon. Nature 314:141-144

Nordman H, Davies JR, Lindell G, de Bolós C, Real F, Carlstedt I (2002) Gastric MUC5AC and MUC6 are large oligomeric mucins that differ in size, glycosylation and tissue distribution. Biochem J 364:191-200

Odagi I, Kato T, Imazu H, Kaise M, Omar S, Tajiri H (2007) Examination of normal intestine using confocal endomicroscopy. J Gastroenterol Hepatol 22:658-662

Offner GD, Troxler RF (2000) Heterogeneity of high-molecular-weight human salivary mucins. Adv Dent Res 14:69-75

Olmsted SS, Padgett JL, Yudin AI, Whaley KJ, Moench TR, Cone RA (2001) Diffusion of macromolecules and virus-like particles in human cervical mucus. Biophys J 81:1930-1937

Ouwehand AC, Isolauri E, Kirjavainen PV, Salminen SJ (1999) Adhesion of four Bifidobacterium strains to human intestinal mucus from subjects in different age groups. FEMS Microbiol Lett 172:61-64

Palmai-Pallag T, Khodabukus N, Kinarsky L, Leir S, Sherman S, Hollingsworth M, Harris A (2005) The role of the SEA (sea urchin sperm protein, enterokinase and agrin) module in cleavage of membrane-tethered mucins. Febs J 272:2901-2911

Pappenheimer JR (1988) Physiological regulation of epithelial junctions in intestinal epithelia. Acta Physiol Scand Suppl 571:43

Pappenheimer JR (1990) Paracellular intestinal absorption of glucose, creatinine, and mannitol in normal animals: relation to body size. Am J Physiol 259:G290-299

Pappenheimer JR (1998) Scaling of dimensions of small intestines in non-ruminant eutherian mammals and its significance for absorptive mechanisms. Comp Biochem Physiol A 121:45-58

Pappenheimer JR (2001a) Intestinal absorption of hexoses and amino acids: from apical cytosol to villus capillaries. J Membr Biol 184:233-239

Pappenheimer JR (2001b) Role of pre-epithelial 'unstirred' layers in absorption of nutrients from the human jejunum. J Membr Biol 179:185-204

Pappenheimer JR, Dahl CE, Karnovsky ML, Maggio JE (1994) Intestinal absorption and excretion of octapeptides composed of D amino acids. Proc Natl Acad Sci USA 91:1942-1945

Pappenheimer JR, Reiss KZ (1987) Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. J Membr Biol 100:123-136

Pedley KC, Naftalin RJ (1993) Evidence from fluorescence microscopy and comparative studies that rat, ovine and bovine colonic crypts are absorptive. J Physiol 460:525-547

Peppas NA, Hansen PJ, Buri PA (1984) A theory of molecular diffusion in the intestinal mucus. Int J Pharm 20:107-118

Phillips A, France N, Walker-Smith J (1979) The structure of the enterocyte in relation to its position on the villus in childhood: an electron microscopical study. Histopathology 3:117-130

Pohl P, Saparov SM, Antonenko YN (1998) The size of the unstirred layer as a function of the solute diffusion coefficient. Biophys J 75:1403-1409

Poulsen LK, Lan F, Kristensen CS, Hobolth P, Molin S, Krogfelt KA (1994) Spatial distribution of *Escherichia coli* in the mouse large intestine inferred from rRNA in situ hybridization. Infect Immun 62:5191-5194

Powell DW (1981) Barrier function of epithelia. Am J Physiol 241:G275-288

Powell DW (1986) Ion and water transport in the intestine. In: Andreoli TE, Hoffman JF, Faunstial DO, Schultz SG (eds) Physiology of membrane disorders. Plenum, New York, pp 559-596

Pries AR, Secomb TW, Gaehtgens P (2000) The endothelial surface layer. Pflügers Arch 440:653-666

Pries AR, Secomb TW, Jacobs H, Sperandio M, Osterloh K, Gaehtgens P (1997) Microvascular blood flow resistance: role of endothelial surface layer. Am J Physiol 273:H2272-2279

Ranc H, Elkhyat A, Servais C, Mac-Mary S, Launay B, Humbert P (2006) Friction coefficient and wettability of oral mucosal tissue: Changes induced by a salivary layer. Colloid Surf A Physicochem Eng Aspects 276:155-161

Raviv U, Klein J (2002) Fluidity of bound hydrate layers. Science 297:1540–1543

Read NW, Levin RJ, Holdsworth CD (1976) Proceedings: Measurement of the functional unstirred layer thickness in the human jejunum *in vivo*. Gut 17:387

Rees WD, Turnberg LA (1982) Mechanisms of gastric mucosal protection: a role for the 'mucus-bicarbonate' barrier. Clin Sci 62:343-348

Rijnaarts HHM, Norde W, Bouwer EJ, Lyklema J, Zehnder AJB (1993) Bacterial adhesion under static and dynamic conditions. Appl Environ Microbiol 59:3255-3265

Robbe C, Capon C, Maes E, Rousset M, Zweibaum A, Zanetta JP, Michalski JC (2003) Evidence of regio-specific glycosylation in human intestinal mucins. J Biol Chem 278:46337-46348

Robert C, Bernalier-Donadille A (2003) The cellulolytic microflora of the human colon: evidence of microcrystalline cellulose degrading bacteria in methane excreting subjects. FEMS Microbiol Ecol 46:81-89

Rodrigueza W, Thuahnai S, Temel R, Lund-Katz S, Phillips M, Williams D (1999) Mechanism of scavenger receptor class B type I-mediated selective uptake of cholesteryl esters from high density lipoprotein to adrenal cells. J Biol Chem 274:20344

Rojas M, Ascencio F, Conway PL (2002) Purification and characterization of a surface protein from *Lactobacillus fermentum* 104R that binds to porcine small intestinal mucus and gastric mucin. Appl Environ Microbiol 68:2330-2336

Rose MC, Voynow JA (2006) Respiratory tract mucin genes and mucin glycoproteins in health and disease. Physiol Rev 86:245-278

Ross GA, Mayhew TM (1985) Effects of fasting on mucosal dimensions in the duodenum, jejunum and ileum of the rat. J Anat 142:191-200

Ryu K, Grim E (1982) Unstirred water layer in canine jejunum. Am J Physiol 242:G364-369

Salas PJI, Moreno JH (1982) Single-file diffusion multi-ion mechanism of permeation in paracellular epithelial channels. J Membr Biol 64:103-112

Saltzman WM, Radomsky ML, Whaley KJ, Cone RA (1994) Antibody diffusion in human cervical mucus. Biophys J 66:508-515

Sandzen B, Blom H, Dahlgren S (1988) Gastric Mucus Gel Layer Thickness Measured by Direct Light Microscopy: An Experimental Study in the Rat. Scand J Gastroenterol 23:1160-1164

Sarosiek J, Marshall BJ, Peura DA, Hoffman S, Feng T, McCallum RW (1991) Gastroduodenal mucus gel thickness in patients with *Helicobacter pylori*: a method for assessment of biopsy specimens. Am J Gastroenterol 86:729-734

Savidge TC, Smith MW, Mayel-Afshar S, Collins AJ, Freeman TC (1994) Selective regulation of epithelial gene expression in rabbit Peyer's patch tissue. Pflügers Arch 428:391-399

Sawaguchi A, Ishihara K, Kawano J, Oinuma T, Hotta K, Suganuma T (2002) Fluid dynamics of the excretory flow of zymogenic and mucin contents in rat gastric gland processed by high-pressure freezing/freeze substitution. J Histochem Cytochem 50:223-234

Schafer JA, Andreoli TE (1986) Principles of water and nonelectrolyte transport across membranes. In: Andreoli TE, Hoffman JF, Fanestil DD, Schultz SG (eds) Physiology of Membrane Disorders. Plenum, New York, pp 177-190

Schneider WR, Doetsch RN (1974) Effect of viscosity on bacterial motility. J Bacteriol 117:696-701

Schultsz C, van den Berg FM, ten Kate FW, Tytgat GNJ, Dankert J (1999) The intestinal mucus layer from patients with inflammatory bowel disease harbors high numbers of bacteria compared with controls. Gastroenterology 117:1089-1097

Sellers LA, Allen A, Morris ER, Ross-Murphy SB (1991) The rheology of pig small intestinal and colonic mucus: weakening of gel structure by non-mucin components. Biochim Biophys Acta 1115:174-179

Semenza G (1986) Anchoring and biosynthesis of stalked brush border membrane proteins: glycosidases and peptidases of enterocytes and renal tubuli. Annu Rev Cell Biol 2:255-307

Sheahan DG, Jervis HR (1976) Comparative histochemistry of gastrointestinal mucosubstances. Am J Anat 146:103-131

Shen L, Turner JR (2006) Role of epithelial cells in initiation and propagation of intestinal inflammation. Eliminating the static: tight junction dynamics exposed. Am J Physiol 290:G577-582

Shogren R, Gerken TA, Jentoft N (1989) Role of glycosylation on the conformation and chain dimensions of O-linked glycoproteins: light-scattering studies of ovine submaxillary mucin. Biochemistry 28:5525-5536

Silver DL, Wang N, Xiao X, Tall AR (2001) High density lipoprotein (HDL) particle uptake mediated by scavenger receptor class B type 1 results in selective sorting of HDL cholesterol from protein and polarized cholesterol secretion. J Biol Chem 276:25287-25239

Smart J (2005) The basics and underlying mechanisms of mucoadhesion. Adv Drug Deliv Rev 57:1556-1568

Smith GW, Wiggins PM, Lee SP, Tasman-Jones C (1986) Diffusion of butyrate through pig colonic mucus in vitro. Clin Sci 70:271-276

Smithson KW, Millar DB, Jacobs LR, Gray GM (1981) Intestinal diffusion barrier: unstirred water layer or membrane surface mucous coat? Science 214:1241-1244

Snoeck V, Goddeeris B, Cox E (2005) The role of enterocytes in the intestinal barrier function and antigen uptake. Microbes and Infection 7:997-1004

Specian RD, Oliver MG (1991) Functional biology of intestinal goblet cells. Am J Physiol 260:C183-193

Stahl A (2004) A current review of fatty acid transport proteins (SLC27). Pflügers Arch 447:722-727

Stein WD (1967) The movement of molecules across cell membranes. Academic Press, New York

Stern M, Walker WA (1984) Food proteins and gut mucosal barrier I. Binding and uptake of cow's milk proteins by adult rat jejunum *in vitro*. Am J Physiol 246:G556-562

Strocchi A, Levitt MD (1993) Role of villous surface area in absorption science versus religion. Dig Dis Sci 38:385-387

Strous GJ, Dekker J (1992) Mucin-type glycoproteins. Crit Rev Biochem Mol Biol 27:57-92

Su F, Brands R, Wang Z, Verdant C, Bruhn A, Cai Y, Raaben W, Wulferink M, Vincent JL (2006) Beneficial effects of alkaline phosphatase in septic shock. Crit Care Med 34:2182-2187

Swidsinski A, Loening-Baucke V, Theissig F, Engelhardt H, Bengmark S, Koch S, Lochs H, Doerffel Y (2007a) Comparative study of the intestinal mucus barrier in normal and inflamed colon. Gut 56:343-350

Swidsinski A, Sydora BC, Doerffel Y, Loening-Baucke V, Vaneechoutte M, Lupicki M, Scholze J, Lochs H, Dieleman LA (2007b) Viscosity gradient within the mucus layer determines the mucosal barrier function and the spatial organization of the intestinal microbiota. Inflamm Bowel Dis 13:963-970

Tajima K, Aminov RI, Nagamine T, Ogata K, Nakamura M, Matsui H, Benno Y (1999) Rumen bacterial diversity as determined by sequence analysis of 16S rDNA libraries. FEMS Microbiol Ecol 29:159-169

Taylor C, Allen A, Dettmar PW, Pearson JP (2003) The gel matrix of gastric mucus is maintained by a complex interplay of transient and nontransient associations. Biomacromolecules 4:922-927

Taylor C, Allen A, Dettmar PW, Pearson JP (2004) Two rheologically different gastric mucus secretions with different putative functions. Biochim Biophys Acta 1674:131-138

Taylor C, Draget KI, Pearson JP, Smidsrød O (2005) Mucous systems show a novel mechanical response to applied deformation. Biomacromolecules 6:1524-1530

Terra WR (2001) The origin and functions of the insect peritrophic membrane and peritrophic gel. Arch Insect Biochem Physiol 47:47-61

Thomson AB, Dietschy JM (1977) Derivation of the equations that describe the effects of unstirred water layers on the kinetic parameters of active transport processes in the intestine. J Theor Biol 64:277-294

Tirosh B, Rubinstein A (1998) Migration of adhesive and nonadhesive particles in the rat intestine under altered mucus secretion conditions. J Pharm Sci 87:453-456

Travis S, Menzies I (1992) Intestinal permeability: functional assessment and significance. Clin Sci 82:471-488

Tsai HH, Dwarakanath AD, Hart CA, Milton JD, Rhodes JM (1995) Increased faecal mucin sulphatase activity in ulcerative colitis: a potential target for treatment. Br Med J 36:570-576

Turner JR, Rill BK, Carlson SL, Carnes D, Kerner R, Mrsny RJ, Madara JL (1997) Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. Am J Physiol 273:C1378-1385

Turner NC, Martin GP, Marriott C (1985) The influence of native porcine gastric mucus gel on hydrogen ion diffusion: the effect of potentially ulcerogenic agents. J Pharm Pharmacol 37:776-780

Ugolev A (1989) Membrane digestion: new facts and concepts. Mir Publishers

Ugolev AM (1962) Membrane digestion. Bull Exp Biol Med 52:874-877

Ugolev AM, De Laey P (1973) Membrane digestion a concept of enzymic hydrolysis on cell membranes. Biochim Biophys Acta 300:105-128

Ugolev AM, Smirnova LF, Iezuitova NN, Timofeeva NM, Mityushova NM, Egorova VV, Parshkov EM (1979) Distribution of some adsorbed and intrinsic enzymes between the mucosal cells of the rat small intestine and the apical glycocalyx separated from them. FEBS Lett 104:35-38

Van der Waaij LA (2003) The interaction between the mucosal immune system and the commensal microflora of the colon. Phd Thesis, Rijksuniversiteit, Groningen

van Veen SQ, van Vliet AK, Wulferink M, Brands R, Boermeester MA, van Gulik TM (2005) Bovine Intestinal Alkaline Phosphatase Attenuates the Inflammatory Response in Secondary Peritonitis in Mice. Infect Immun 73:4309-4314

Vecchi M, Torgano G, Monti M, Berti E, Agape D, Primignani M, Ronchi G, Franchis R (1987) Evaluation of structural and secretory glycoconjugates in normal human jejunum by means of lectin histochemistry. Histochem Cell Biol 86:359-364

Velez M, De Keersmaecker S, Vanderleyden J (2007) Adherence factors of Lactobacillus in the human gastrointestinal tract. FEMS Microbiol Lett 276:140-148

Verdugo P (1990) Goblet cells secretion and mucogenesis. Annu Rev Physiol 52:157-176

Veerman EC, Valentijn-Benz M, Nieuw Amerongen AV (1989) Viscosity of human salivary mucins: Effect of pH and ionic strength and role of sialic acid. J Biol Buccale 17:297-306

Waheed AA, Gupta PD (1997) Changes in structural and functional properties of rat intestinal brush border membrane during starvation. Life Sci 61:2425-2433

Waigh TA, Papagiannopoulos A, Voice A, Bansil R, Unwin AP, Dewhurst CD, Turner B, Afdhal N (2002) Entanglement Coupling in Porcine Stomach Mucin. Langmuir 18:7188-7195

Walker AW, Duncan SH, Harmsen HJM, Holtrop G, Welling GW, Flint HJ (2008) The species composition of the human intestinal microbiota differs between particle associated and liquid phase communities. Environ Microbiol 10:3275-3283

Watson CJ, Rowland M, Warhurst G (2001) Functional modeling of tight junctions in intestinal cell monolayers using polyethylene glycol oligomers. Am J Physiol 281:C388-397

Westergaard H, Dietschy JM (1974) Delineation of the dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine. J Clin Invest 54:718-732

Westergaard H, Holtermuller KH, Dietschy JM (1986) Measurement of resistance of barriers to solute transport in vivo in rat jejunum. Am J Physiol 250:G727-735

Winne D (1978) Dependence of intestinal absorption *in vivo* on the unstirred layer. Naunyn Schmiedebergs Arch Pharmacol 304:175-181

Winne D (1979) Rat jejunum perfused in situ: effect of perfusion rate and intraluminal radius on absorption rate and effective unstirred layer thickness. Naunyn Schmiedebergs Arch Pharmacol 307:265-274

Winne D (1989) Effect of villosity and distension on the absorptive and secretory flux in the small intestine. J Theor Biol 139:155-186

Winne D, Kopf S, Ulmer ML (1979) Role of unstirred layer in intestinal absorption of phenylalanine *in vivo*. Biochim Biophys Acta 550:120-130

Womack WA, Mailman D, Kvietys PR, Granger DN (1988a) Neurohumoral control of villous motility. Am J Physiol 255:G162-167

Womack WA, Tygart PK, Mailman D, Kvietys PR, Granger DN (1988b) Villous motility: relationship to lymph flow and blood flow in the dog jejunum. Gastroenterology 94:977-983

Yakubov GE, McColl J, Bongaerts JH, Ramsden JJ (2009) Viscous Boundary Lubrication of Hydrophobic Surfaces by Mucin. Langmuir 25:2313-2321.

Yakubov GE, Papagiannopoulos A, Rat E, Waigh TA (2007) Charge and interfacial behavior of short side-chain heavily glycosylated porcine stomach mucin. Biomacromolecules 8:3791-3799

Yang DH, Kasamo H, Miyauchi M, Tsuyama S, Murata F (1996) Ontogeny of sulphated glycoconjugate-producing cells in the rat fundic gland. Histochem J 28:33-43

Ymele-Leki P, Ross JM (2007) Erosion from *Staphylococcus aureus* biofilms grown under physiologically relevant fluid shear forces yields bacterial cells with reduced avidity to collagen. Appl Environ Microbiol 73:1834-1841

Young KD (2006) The selective value of bacterial shape. Microbiol Mol Biol Rev 70:660-703

Zeuthen T (1983) Ion activities in the lateral intercellular spaces of gallbladder epithelium transporting at low external osmolarities. J Membr Biol 76:113-122

Zeuthen T (2010) Water-Transporting Proteins. J Membr Biol 234:57-73

Zeuthen T, Hamann S, La Cour M (1996) Cotransport of H^+, lactate and H_2O by membrane proteins in retinal pigment epithelium of bullfrog. J Physiol 497:3-17

Zeuthen T, Zeuthen E, MacAulay N (2007) Water transport by GLUT2 expressed in *Xenopus laevis* oocytes. J Physiol 579:345-361

Zhao J, Liao D, Yang J, Gregersen H (2003) Viscoelastic behavior of small intestine in streptozotocin-induced diabetic rats. Dig Dis Sci 48:2271-2277

Zheng D, Taylor GT, Gyananath G (1994) Influence of laminar flow velocity and nutrient concentration on attachment of marine bacterioplankton. Biofouling 8:107-120

Index

R. G. Lentle, P. W. M. Janssen, *The Physical Processes of Digestion*,
DOI 10.1007/978-1-4419-9449-3,