are not necessarily mutually exclusive, and may operate on different time scales (Applebaum 1985). Unfortunately, experimentally distinguishing these types of control is difficult. The best evidence for hormonal influences comes from mosquitoes (Lehane *et al.* 1995). Because the term 'secretagogue' can be confusing, the latter authors have proposed that direct interaction of a component of the meal with enzyme-producing cells should be termed a prandial mechanism. They also distinguish between paracrine and endocrine mechanisms: a paracrine effect is a local hormonal effect on neighbouring cells. The diffuse endocrine system of the insect midgut remains a major obstacle to differentiating between prandial and endocrine control.

Many studies have investigated the effect of proteins on protease levels in haematophagous Diptera, because of their large and infrequent blood meals, and their role as disease vectors. Diverse soluble proteins stimulate trypsin secretion into the incubation medium of midgut homogenates of the stable fly *Stomoxys calcitrans* (Diptera, Muscidae), and the effect is concentration-dependent (Blakemore *et al.* 1995). This method distinguishes between effects on synthesis and on secretion, because new synthesis over the time scale of the incubations is considered negligible. Insoluble proteins, small peptides and amino acids do not stimulate trypsin secretion. Regulation of levels can vary within an enzyme family. In female *Anopheles gambiae* (Diptera, Culicidae), for example, some trypsins are constitutively expressed, while others, produced in larger amounts, are induced by blood feeding (Müller *et al.* 1995). Rapid advances at the molecular level are being driven by interest in the regulation of serine proteinases of blood-sucking insects (Lehane *et al.* 1995). This interest extends to midgut immunity through the recently discovered defensin family of peptides. Hamilton *et al.* (2002) have shown that midgut defensins of *S. calcitrans* are colocalized with a serine proteinase during storage, and that the complex dissociates on secretion into the lumen, the defensins protecting the stored blood meal from bacterial attack. Insects adapt to proteinase inhibitors in their diet by hyperproduction of proteinases or by switching to novel proteinases that are insensitive to these plant defences (see Section 2.4.3).

Peritrophic matrix and the organization of digestion

The midgut cells of most insects (Hemiptera excluded) secrete a multilayered peritrophic matrix (although frequently called a peritrophic membrane, it lacks cellular structure) consisting of chitin, proteins, and proteoglycans. This functions as a physical barrier to protect the epithelium from mechanical abrasion, toxic plant allelochemicals, and pathogens, and also allows compartmentalization of the gut lumen and the spatial organization of digestive processes. Large macromolecules are hydrolysed by soluble enzymes inside the peritrophic matrix, until they are small enough to diffuse into the space between the peritrophic matrix and midgut epithelium, where digestion is completed by membrane-bound enzymes which may be integral proteins of the microvillar membranes (Terra *et al.* 1996*b*). Structural strength is provided by the meshwork of chitin fibrils, while permeability properties are determined by pore diameters in the gel-like matrix. Labelled dextrans with diameters ranging from 21 to 36 nm penetrate the peritrophic matrix of several species of Lepidoptera and Orthoptera (Barbehenn and Martin 1995), so size exclusion does not explain impermeability to digestive enzymes or to tannins, and other properties of the matrix may be involved.

The importance of midgut compartments in the efficient, sequential breakdown of food was first demonstrated in *Rhynchosciara americana* (Diptera, Sciaridae) by assaying enzyme activities in different luminal compartments and midgut tissue (Terra *et al.* 1979). It is also thought that countercurrent flux of fluid assists in both the absorption of nutrients and the recycling of digestive enzymes. Countercurrent fluxes may result from fluid secretion in the posterior midgut or the anterior movement of primary urine from the Malpighian tubules, the result being that fluid moves in an anterior direction outside the pertriophic matrix, and is absorbed in the anterior midgut or caecae. The anatomical differences vary with phylogeny. The evidence for compartmentalization of digestive processes in the major insect orders has been thoroughly reviewed by Terra and colleagues (Terra 1990; Terra and Ferreira 1994; Terra *et al.* 1996*b*). Countercurrent movement of gut fluids occurs in Orthoptera, but only in animals deprived