An investigation of the structure and function of
the peritropic membrane of the American cockroach, Periplaneta
americana, with special reference to the possible effects of
tannins on water movement and permeability of dyes through the
peritrophic membrane.

by

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The peritrophic membrane (PM) is often said to protect the midgut epithelium from abrasion by solid food particles, or act as a physical barrier to penetration of the midgut epithelium by micro-organisms, or as an ultrafilter. Recently, it has also been widely implicated in the conservation of enzymes by creating an ectoperitrophic space next to the midgut epithelium which allows a flow of solutes, including enzymes, counter to the movement of the ingesta, and concentrates these solutes in the ceca. Other work has also implicated the PM of polyphagous phytophagous insects in protection of the midgut from ingested tannins. The PM in orthopteroid insects (including *Periplaneta americana*) is widely regarded as being "delaminated" from the entire midgut.

The present study briefly reviews this work and investigates the structure, function and permeability of the PM in *P. americana* and the possible effects tannins may have on these parameters.

*P. americana* was used especially because it is a generalist feeder not specifically adapted to dietary tannins; limited comparisons were made with another species of cockroach, *Panesthia cribra* which might encounter tannins due to its wood-eating habit.
Pulse radiolabelling experiments, feeding experiments with coloured diets, and injection of dyes into the abdomen indicated that the bulk of the PM is secreted by cells of the anterior end of the midgut. When a previously fasted roach began to feed a new PM was produced at 5-7 mm/hr and production of the PM could possibly also be influenced by some humoral stimuli.

Feeding experiments and injection of dyes also showed retention of dyes in the caeca, perhaps due to a countercurrent system and/or to other physical concomitants of the interaction of the PM and midgut, including patterns of compaction of food and changing permeability of the PM after it is secreted.

Polyphenols affected the PM in *P. americana* differently *in vitro* and *in vivo*: when PM sections were soaked in solutions of tannate or gallate, polyphenolic depositions could be observed only in acidic conditions. When cockroaches ingested polyphenols in a standard diet containing only starch, sugar and silica, no polyphenolic depositions were seen on the PM although the PM structure was affected. Tannic acid in their diet suppressed consumption and other feeding parameters of the insects but no significant effect of tannic acid on permeability parameters of the PM could be demonstrated.

Electron microscopy of the PM in *P. americana* showed it to be a variform lamellate cylindrical sheath holding the solid
to be a variform lamellate cylindrical sheath holding the solid ingesta. The layers consisted mainly of hexagonally or orthogonally orientated grid systems. The ectoperitrophic surface, however, was differentiated along its length: where it overlaid the oesophageal invagination, the microfibrils appeared disorganised, perhaps reflecting the apparent "fluidity" of the PM in this region. Were also, the PM appeared closely associated with the oesophageal invagination due to numerous small spines, or microtrichia. Posterior to this "fluid" area is a region of hexagonal and/or orthogonal grid systems. From 2-3 mm past the tip of the oesophageal invagination and for the rest of the length of the midgut, these grid systems were overlaid with a largely amorphous ectoperitrophic surface layer, consisting of matted fibrils that were closely associated with the underlying grid system and had their interstices filled with a granular substance (hence the amorphous appearance of the layer). It was this ectoperitrophic surface that was affected by ingested tannic acid which caused an apparent lack of the granular substance, leaving only the matted fibrillar network at the surface.

The hydraulic conductivity of the PM in *F. americana* was found to be 0.091 cm/s.bar by an in vitro blind-sac perfusion technique. Using the same technique, permeability to various dyes was carried out when PM sections were bathed in water. Three charged dyes of formula weight 300 to ca. 1300 Dalton, Azure B, Trypan blue, and Alcian blue, all increased the
hydraulic conductivity, but a protein-dye conjugate, Azoalbumin, with a formula weight of ca. 7 x 10^6 Dalton, caused an effective blocking of membrane pores and the hydraulic conductivity was reduced to 0.006 cm/s.bar. When the PM sections were bathed in saline, this apparent blockage of the PM was almost totally removed with a ten-fold increase in hydraulic conductivity. In the absence of Azoalbumin, any combination of saline/water either side of the membrane increased hydraulic conductivity by about 150%. Moreover, the effects of the saline were reversible. These results suggest that the PM acts as a microfilter, and as an ultrafilter under some conditions. As a result, solutes and perhaps enzymes, can remain in the midgut for much longer periods than initial passage of particulate ingesta might suggest. It is proposed that the PM subserves digestive processes in a variety of ways, based on its retention and compaction of solid particles within its lumen.