

## **Malpighian tubules and formation of uric acid**

### *Malpighian tubules*

The main excretory organ of the insect is the **Malpighian tubule**. Insects contain anything from 2 to 150 or more Malpighian tubules depending on the species. Malpighian tubules are tubular outgrowths of the gut. They typically develop as pouches emerging from the junction between the midgut and the hindgut, though their actual final position varies - they may be attached to the midgut, hindgut or the midgut-hindgut junction.

Each Malpighian tubule is a blind-ending tube whose lumen is continuous with the lumen of the gut. Each consists of a single layer of epithelial cells, forming the tubule wall, enclosed by an elastic membrane (basement membrane - a fibrous and porous protein mesh). In most insects there is a thin layer of striated muscle around this membrane. Typically muscle cells spiral around the distal end (the end furthest from the gut) of the tubule, causing it to twist and turn in gentle writhing movements as the muscles contract. The proximal end (near the gut) may be coated in circular and longitudinal muscle fibres, giving rise to peristalsis or squeezing movements which empty the contents of the tubule into the gut.

The body cavity of the insect is filled with a fluid, usually colourless, called haemolymph. This fluid bathes the organs and tissues and is circulated around the insect body. The tubules are also typically loosely or firmly anchored in place by the tracheae which attach to them.

The twisting and turning of the Malpighian tubules presumably keeps them in contact with fresh haemolymph (perhaps by circulating the haemolymph around the tubule). Metabolic wastes and other unwanted chemicals that entered the insect system pass into the haemolymph, or are excreted into the haemolymph by the cells. These include nitrogenous waste and plant toxins such as alkaloids. It is the job of the Malpighian tubules to keep the haemolymph cleansed of these wastes - they remove wastes from the haemolymph and then excrete them into the gut lumen.

### *Physiology of excretion*

Waste materials and excess water pass from the haemolymph into the Malpighian tubules, by crossing the epithelial wall of these blind-ended tubes. Recent evidence shows that these cells contain proton pumps which use energy in the form of ATP to pump protons into the lumen of the Malpighian tubule. Protons are positively charged and to maintain charge balance the

removal of protons from the epithelial cells, into the tubule lumen, is balanced by the inward movement of potassium ions, which move from the haemolymph, into the epithelial cells and then out into the tubule lumen also. The epithelial cells have microvilli projecting into the tubule lumen and are rich in mitochondria which produce the ATP required by the pumps. Removal of the protons from the epithelial cell makes the cytoplasm more negatively charged and also sets up a concentration gradient (that is an electrochemical gradient is established) and this attracts positive ions, such as sodium ( $\text{Na}^+$ ) and potassium ( $\text{K}^+$ ) into the cell from the haemolymph. The influx of these positive ions drags in negative chloride ions to balance the charge. These ions move across the cytoplasm of the cell, the so-called **transcellular pathway**. Note the potassium-chloride and sodium-chloride symporters, the proton-potassium and proton-sodium antiporters and the ion channels. The flux of ions across the epithelial cell also draws across water, by osmosis. This probably takes place largely by the **paracellular pathway**, that is between the epithelial cells. Sugars and amino acids are swept along by the water into the tubule lumen. Since these materials are useful they will be reabsorbed later further downstream.

Other small molecules (small enough to cross the basement membrane) will also move into the tubule through this pathway. The transport of a substance which depends directly on ATP, such as the pumping of the protons in the Malpighian tubule, is called **active transport**. The transport of the other ions and water is passive (by facilitated diffusion) in of itself, but is dependent on proton transport and so indirectly dependent on ATP. This mode of transport is called **secondary active transport**, e.g. the transport of potassium.

In dry conditions many insects can produce a very concentrated urine, indeed one that is 'bone-dry'. However, many insects ingest large quantities of water when feeding, such as blood-sucking insects, and in this instance the rate of fluid-flow through the Malpighian tubules increases a thousandfold or more. Indeed, the rate of fluid transport in these tubules is said to be higher, gram for gram, than any other tissue. Two hormones, released into the haemolymph, can stimulate Malpighian tubules to rapidly increase their rate of fluid transport: 5HT (5-hydroxytryptamine) and a peptide hormone. Increased excretion is triggered by an increase in uric acid following a meal, which presumably triggers the release of the diuretic (urine-producing) hormones. Of course, not all the fluid transported through the tubules is excreted. The proximal (basal or lower or downstream) sections of the tubules, along with the hindgut (especially the rectum) reabsorb some of the water, depending on need, and other useful substances, such as certain ions, sugars and amino acids, so as to

produce a final urine of the 'desired' concentration. It is in this proximal or lower part of the tubule that uric acid is transported into the tubule, against a concentration gradient, and precipitates as crystals, e.g. of insoluble potassium urate as the urate combines with the high potassium content of the tubule lumen. In some insects these crystals can be seen filling the lumens of the proximal ends of the tubules. Presumably, peristalsis then moves these crystals along into the gut. Potassium and some of the chloride are recovered in this way, producing urine high in sodium.

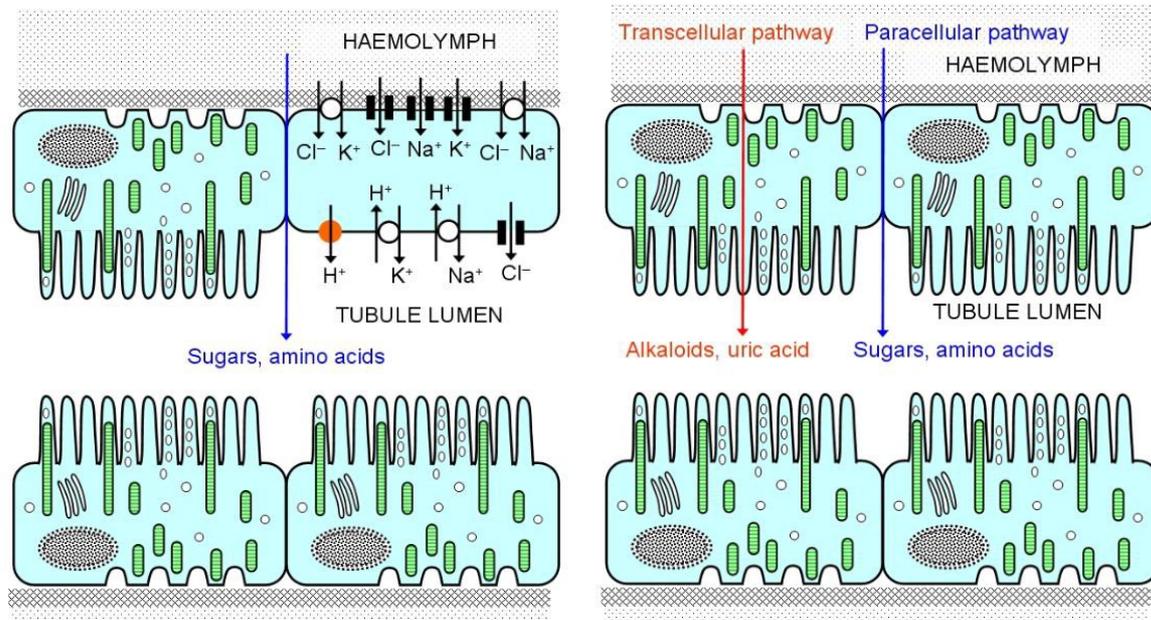


Fig.: Movement of substances from haemolymph into the lumen of malpighian tubules

Some small organic molecules are also actively transported into the tubule lumen by the transcellular pathway, including alkaloids (plant compounds which may be toxic to the insect).

Uric acid, mostly in the form of negatively charged urate ions, is also actively transported by the transcellular pathway, though the exact mechanism is not well understood. This urate transport occurs in the proximal tubule and the urate combines with the potassium transported into the tubule to form insoluble potassium urate crystals. These crystals form roughly spherical concretions in the tubule lumen. The microvilli in the proximal tubule seem to undergo a cycle of elongation, as the urate concretions form, and retraction as the lumen fills up with urate waiting to be transported into the gut. Once in the gut, remaining water may be reabsorbed as needed and the remaining urate excreted with the faeces, or separately. The

midgut is divided from the hindgut by the pyloric sphincter and when this sphincter is closed the hindgut receives only the contents of the Malpighian tubules. The mechanism of excretion demonstrated by the Malpighian tubule is one largely dependant on 'secretion' of unwanted materials, such as urate and excess sodium. This contrasts with the mammalian kidney which relies on ultrafiltration (filtration through microscopic pores), which removes most materials from the blood except large proteins and cells, followed by reabsorption of what the body needs to keep, such as sugars and amino acids. However, there is some filtration in the Malpighian tubule, namely the influx of materials through the paracellular pathway, having filtered across the basement membrane. Sugars and amino acids filtered in this way are then reabsorbed, as in the mammalian case. Similarly, there is some secretion in the mammalian kidney, for example the secretion of protons and ammonium in acid-base balance and the secretion of some drugs such as penicillin. However, the emphasis is different with the Malpighian tubule relying more on secretion, the mammalian kidney on filtration.