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Inside JEB

SIMON MADDRELL AND 50 YEARS OF INSECT HOMEOSTASIS

Simon Maddrell has been synonymous with insect homeostasis since he joined V. B. Wigglesworth's lab as a graduate student in 1959. Fascinated by the remarkable blood-sucking feats of *Rhodnius prolixus*, a rather unpleasant insect that is capable of excreting its own body weight in fluid every 40 minutes, Maddrell has inspired and enthused the generations of insect physiologists that have come into contact with him during his 50 year career.

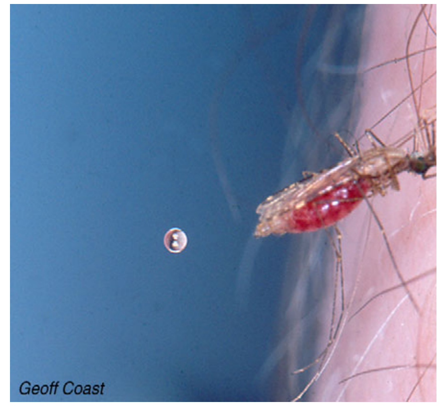
Maddrell's tissue of choice throughout his career has been the Malpighian tubule, which produces primary urine *via* solute and solvent transport across the tubule's cells. Many salts transported into the primary urine are then reabsorbed by the insect's hindgut, allowing insects to excrete large volumes of fluid while maintaining their osmotic balance. Maddrell has focused on identifying many of the hormones responsible for regulating this delicate process.

One of Maddrell's many acolytes, Julian Dow, remembers being inspired by Maddrell's final year Zoology undergraduate lectures at the University of Cambridge in the 1970s. 'His lectures really stood out' says Dow and he was lucky enough to join Maddrell's lab in 1977 as a graduate student to work on fluid movements involved in digestion in the desert locust. 'Simon had a relaxed style' remembers Dow, 'he left his students to do their own thing, but reappeared regularly to enthuse us' he adds.

Five decades on, 'Maddrell is finally beginning to hang up his research boots' says Dow and adds that it seemed fitting to acknowledge Maddrell's pivotal influence on the field of insect homeostasis. Teaming up with Glasgow colleague, Shireen-Anne Davies, Dow has invited many of Maddrell's friends and colleagues to contribute to this special collection of papers in *The Journal of Experimental Biology* dedicated to Maddrell and his extraordinary contribution to the field of insect homeostasis.

SOLUTE AND SOLVENT TRANSPORT MECHANISMS

Over the last 20 years, Klaus Beyenbach has focused on secretion mechanisms in the Malpighian tubules of *Aedes aegypti*, the yellow fever mosquito. Curious to know more about chloride ion and water transport *via* paracellular pathways (the narrow junctions between cells in the Malpighian tubule wall), Beyenbach and his team from Cornell University exposed *Aedes*


Geoff Coast

Malpighian tubules to a diuretic hormone to find out which proteins are expressed during chloride and water excretion. They found that expression of two subunits of the energizing V-ATPase and a regulatory subunit of protein kinase A were reduced in the cell's cytosol. This suggests that the energizing proton pump relocates from the cytosol to the cell membrane and that protein kinase A may be involved in the pump's assembly during chloride and water transport between cells (p. 329).

Moving on to one of the key proteins involved in a wide range of transport processes, the proton-pumping transport-energizing V-ATPase, Helmut Wieczorek and Markus Huss discuss the identification and uses of compounds that actively inhibit the energizing enzyme. Huss and Wieczorek explain that apart from powering transport in the insect Malpighian tubule, V-ATPases are known to play a role in a wide number of diseases, including osteoporosis and cancer, making them an attractive target for the development of inhibitory drugs. The duo go on to explain that having identified a drug, it is essential to understand where the drug binds to the V-ATPase with the aim of developing better therapies. They describe how the earliest V-ATPase inhibitors, bafilomycin and concanamycin, are known to inhibit the enzyme by binding to the c subunit of the enzyme's membrane-bound proton translocating V_o subunit. They also outline an extensive list of novel inhibitors, which includes macrolactone archazolid, the benzolactone enamides and novel indole derivatives, outlining what is known about how the drugs bind V-ATPases and their potential roles in cancer therapy (p. 341).

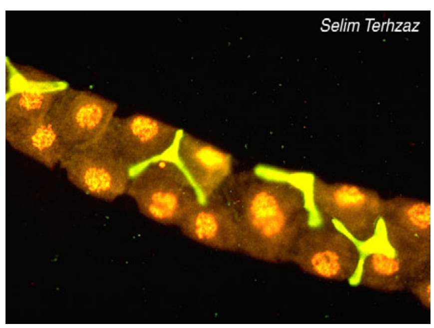
With almost 60 years of experience in the field of solute transport in insect tissues, Bill Harvey discusses the role of amino acid transporters in sodium transport in the mosquito gut. Harvey explains that acid is expelled from cells *via* sodium/hydrogen exchangers and antiporter molecules in the membrane of cells bordering the midgut lumen. While the human genome contains 10 sodium/hydrogen exchangers and two antiporters, mosquitoes only appear to have three exchangers and two antiporters. Realising that the mosquito genome has at least 12 nutrient amino acid transporters that co-transport sodium ions, Harvey suspected that they might compensate for the insect's low number of sodium/hydrogen exchanger genes. Knowing that the amino acid transporters are voltage driven and occur in the same membranes as hydrogen ion transporting V-ATPases, Harvey describes how the membrane potential generated by the V-ATPases drives the sodium linked amino acid transport through the amino acid transporters, providing a route for sodium ion/hydrogen ion exchange that effectively substitutes for the insect's missing sodium ion/hydrogen ion exchangers (p. 347).

While solute transport is essential for many blood-sucking and xylem-feeding insects to regulate their ionic balance, they must also be able to rapidly excrete huge volumes of water while processing a meal. Jeffrey Spring and his colleagues from universities in Louisiana explain that Arthur Ramsay first suggested that water transport across insect Malpighian tubules must be active to move water against an osmotic gradient to produce urine that is more dilute than the insect's haemolymph. It is only in the last 15 years that water has been shown to be transported across cell membranes down a shallow osmotic gradient by proteins known as aquaporins. While 13 aquaporins have been identified in the human genome, only seven have been found in insects, of which four are known to be involved in excretion. Outlining the structure of aquaporins, Spring explains how the water channels block proton leak by reorienting water molecules in the channel and selectively filter water molecules through a narrow channel lined with aromatic and arginine amino acids (p. 358).

Following Spring's discussion of aquaporins, Mike O'Donnell from McMaster University, Canada, explains how blood-feeding insects must also rid themselves of a range of toxic materials ingested with the meal. Having described the mechanisms involved in regulating sodium and chloride levels, O'Donnell goes on to discuss how insects regulate their potassium and calcium levels, as well as dealing with toxic nitrogenous waste and reactive oxygen species. O'Donnell then outlines the electrophysiological techniques essential for the study of toxin transport in Malpighian tubules, before focusing on the effects of toxins, such as willow-derived salicylate, on excretion. Concluding his review, O'Donnell is optimistic that tools such as FlyAtlas, which maps gene expression patterns in adult *Drosophila*, will hopefully teach us more about the molecular mechanisms underpinning toxin excretion in insects (p. 363).

Moving on from toxin management, Horst Onken and David Moffett discuss possible mechanisms that produce the extraordinarily alkaline environment found in the mosquito larvae anterior midgut. According to Onken, the midgut of many lepidopteran and dipteran larvae is one of the most alkaline environments known to biology, ranging from pH 10.5 to 12. It is also well established that this alkalinization in the midgut is driven by V-ATPases in the midgut basal membrane (cell membrane that faces the haemolymph). Having described two potential mechanisms in the apical membrane (the cell membrane that faces the gut lumen) that could account for the anterior midgut pH, one dominated by anion transport and the other dominated by cation transport, Onken and Moffett outline work from their labs that leads them to suggest that 'We are now in a position to rule out or at least cast in serious doubt most elements of the current reasonable hypotheses about the apical membrane transport in this process.' However, they emphasize that they cannot rule out the role of alternative acid–base transport processes in the midgut's alkalinization and add 'the studies analyzed here provide both limitation and direction for further study of the mechanism of strong alkalinization in this system' (p. 373).

NEUROENDOCRINE CONTROL AND DEVELOPMENT



Having extensively reviewed our knowledge and understanding of the molecular mechanisms involved in insect homeostasis, this collection moves on to discuss what is known about the control of transport processes essential for effective homeostasis. Geoff Coast from Birkbeck (University of London), UK, describes the physiological processes associated with the consumption of a blood meal by *Rhodnius prolixus*. He also discusses the major hormones that regulate transport in the Malpighian tubule, such as the diuretic hormone, identified by Maddrell in the early 1960s (serotonin) and the antidiuretic hormone CAP_{2b}, which terminates diuresis 3–4 hours after feeding. Coast then discusses the way that female mosquitoes deal with a blood meal that is 2–3 times their own body weight by simultaneously processing it while gorging. According to Coast, mosquito diuresis is controlled during the early stages by the hormone Mosquito Natriuretic Peptide, excreting large amounts of sodium through the Malpighian tubule before the insect switches to a potassium excretion during the later stages of diuresis, which is controlled by a hormone with a non-selective effect on cation transport (p. 378).

After Coast's discussion of hormones in diuresis, Shireen-A. Davies and Selim Terhzaz consider the role of calcium, which is a second messenger involved in cell homeostasis. Shireen-A. Davies and Selim Terhzaz describe current techniques that are used to probe tissue and cell calcium signals and outline their use in the study of calcium signalling in *Drosophila* Malpighian tubules. Using these molecular approaches, Davies and Terhzaz describe calcium signalling in the organelles of principal cells (one of the two major cell types found in the tubule) and the calcium signalling pathways that modulate fluid transport through the principal cells in response to the capa diuretic hormones. They also discuss calcium transport into (intracellular calcium transport) and calcium storage within principal cells for use during signalling. Davies and Terhzaz add that although calcium signalling clearly modulates fluid transport by Malpighian tubules, it may also play a role in tubule immune sensing and detoxification (p. 387).

Shifting focus from the Malpighian tubule to the hindgut, where insects reabsorb essential ions and water from the Malpighian tubule's primary urine, Heinrich Dircksen outlines the role of the ion transport peptide hormone in chloride transport and acid secretion in the hindgut. Explaining that the hormone can be found in several isoforms, he discusses various neural locations where the peptides are produced and suggests that the longer inactive form of the hormone could function as an inhibitor of the shorter active form. Dircksen concludes by drawing a parallel between insect ion transporting peptide and crustacean hyperglycaemic hormones (which functions in ion transport, water uptake and acid secretion in crustaceans), speculating that cells that produce both peptides may 'share common phylogenetic ancestry' (p. 401).

Having discussed neuroendocrine regulation of excretion in both the Malpighian tubule and hindgut, this collection of papers moves on to discuss development and repair of *Drosophila* Malpighian tubules and parallels that can be drawn with the mammalian kidney. Shree Ram Singh and Steven Hou from the National Cancer Institute at Frederick explain how they have identified multipotent stem cells in *Drosophila* Malpighian tubules. Outlining the developmental processes that must be undergone to produce a complex mammalian kidney, Singh and Hou point out the similarities to the development and function of the *Drosophila* Malpighian tubule. They then explain the role of adult stem cells in kidney repair and tissue turnover, although stem cells have not been identified in the adult kidney. However, Singh and Hou discovered multipotent stem cells in the adult *Drosophila* Malpighian tubule in 2007 and confirmed that a major stem cell signalling pathway is active in the cells, making the *Drosophila* Malpighian tubule an excellent model for development and repair in the human kidney (p. 413).

ORGANISMAL AND POST-GENOMIC VIEWS ON OSMOREGULATION


Michael Ellis

Switching scale from the tissue level to the whole organism, Heidy Contreras and Timothy Bradley from the University of California, Irvine, focus on insect respiration. Specifically, they address a long-standing question in insect physiology: why insects adopt various gas exchange patterns during respiration. First, Contreras and Bradley outline several hypotheses that have been put forward to explain the respiration pattern, such as the Hygric Hypothesis (where discontinuous ventilation limits water loss), the Chthonic Hypothesis (where the breathing pattern may be beneficial during life stages spent in hypoxic subterranean environments) and the Oxidative Damage Hypothesis (where the breathing pattern naturally lowers internal oxygen levels to protect the insect from oxidative damage). Focusing on the third hypothesis, the duo present evidence that *Rhodnius prolixus* adjusts its breathing pattern according to the amount of oxygen that the insects' tracheal system contains when the spiracles close, consistent with the Oxidative Damage Hypothesis (p. 424).

Continuing with the water homeostasis theme, Susan Nicolson from the University of Pretoria addresses insect hydration from the perspective of individual bees and the entire colony. Explaining that bees have a high water turnover compared with other terrestrial insects, due to their nectar diet and the metabolic generation of water by muscle during flight, Nicolson discusses the nectar requirements of the individual and colony and how bees process nectar into honey by sucrose hydrolysis and evaporation. According to Nicolson, evaporation can also serve to cool the individual during flight or cool the entire colony. Nicolson continues by discussing how bees regurgitate nectar from the crop onto the proboscis to allow it to evaporate and concentrate and concludes by saying that this approach to concentrating the nectar 'may have a profound influence on the water balance physiology of solitary bees in addition to social homeostasis' (p. 429).

Bringing the Malpighian tubule squarely into the 21st Century, Julian Dow discusses new research opportunities that recently became possible in the post-genomic era. Outlining many of the modern techniques, such as microarray analysis, currently used routinely in *Drosophila*, Dow describes how they have led to the identification of specific genes that underlie many processes in the Malpighian tubule. These techniques have also allowed scientists to identify new processes that the Malpighian tubule was previously not known to participate in, such as the immune response and DDT detoxification. Dow continues by making the case for the *Drosophila* Malpighian tubule as a potentially powerful model for human kidney diseases, including kidney stones and concludes that 'it is exciting that so many new avenues are being opened by postgenomic techniques,' (p. 435)

IN CONCLUSION

Finally, the man who inspired this review collection, Simon Maddrell, presents his perspective on hormonal regulation of the *Rhodnius prolixus* Malpighian tubule (p. 446). Describing the insect's remarkable ability to consume blood meals 12 times its own unfed weight, Maddrell says 'Perhaps the most interesting problem in *Rhodnius* has to do with how the rate of fluid flow into the haemolymph from the midgut is matched by the rate of fluid flow out of the haemolymph *via* the Malpighian tubules.' Maddrell explains that the matching is achieved by natural dilution of the diuretic hormones in the haemolymph. According to Maddrell, the haemolymph volume increases as the Malpighian tubules' excretion rate drops, instantly lowering the haemolymph hormone level and slowing the rate of hindgut fluid transport. However, the Malpighian tubules, which are significantly more sensitive to diuretic hormones than the midgut, continue producing urine as the hindgut's transport rate drops to match it.

Maddrell concludes by discussing questions that are yet to be answered, and summarizes the little that is currently known about Malpighian tubule transport of inorganic and organic solutes. He is optimistic that 'we can look forward in the next few years to a greatly increased knowledge of all aspects of insect homeostasis,' and concludes that 'Exciting times lie ahead!'

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