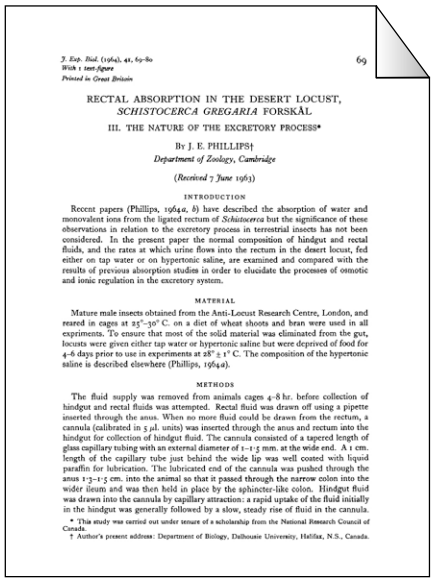
JEB Classics

ACTIVE TRANSPORT IN INSECT RECTA



J. Exp. Biol. (1964), 41, 69–80
With 1 text-figure
Printed in Great Britain

69

RECTAL ABSORPTION IN THE DESERT LOCUST, *SCHISTOCERCA GREGARIA* FORSKÅL

III. THE NATURE OF THE EXCRETORY PROCESS*

By J. E. PHILLIPS†

Department of Zoology, Cambridge

(*Received 7 June 1963*)

INTRODUCTION

Recent papers (Phillips, 1964*a*, *b*) have described the absorption of water and monovalent ions from the ligated rectum of *Schistocerca* but the significance of these observations in relation to the excretory process in terrestrial insects has not been considered. In the present paper the normal composition of hindgut and rectal fluids, and the rates at which urine flows into the rectum in the desert locust, fed either on tap water or on hypertonic saline, are examined and compared with the results of previous absorption studies in order to elucidate the processes of osmotic and ionic regulation in the excretory system.

MATERIAL

Mature male insects obtained from the Anti-Locust Research Centre, London, and reared in cages at 25°–30° C. on a diet of wheat shoots and bran were used in all expriments. To ensure that most of the solid material was eliminated from the gut, locusts were given either tap water or hypertonic saline but were deprived of food for 4–6 days prior to use in experiments at 28° ± 1° C. The composition of the hypertonic saline is described elsewhere (Phillips, 1964*a*).

METHODS

The fluid supply was removed from animals cages 4–8 hr. before collection of hindgut and rectal fluids was attempted. Rectal fluid was drawn off using a pipette inserted through the anus. When no more fluid could be drawn from the rectum, a cannula (calibrated in 5 µl. units) was inserted through the anus and rectum into the hindgut for collection of hindgut fluid. The cannula consisted of a tapered length of glass capillary tubing with an external diameter of 1·1·5 mm. at the wide end. A 1 cm. length of the capillary tube just behind the wide lip was well coated with liquid paraffin for lubrication. The lubricated end of the cannula was pushed through the anus 1·5–1·5 cm. into the animal so that it passed through the narrow colon into the wider ileum and was then held in place by the sphincter-like colon. Hindgut fluid was drawn into the cannula by capillary attraction: a rapid uptake of the fluid initially in the hindgut was generally followed by a slow, steady rise of fluid in the cannula.

* This study was carried out under tenure of a scholarship from the National Research Council of Canada.

† Author's present address: Department of Biology, Dalhousie University, Halifax, N.S., Canada.

Timothy Bradley discusses John Phillips's 1964 paper: Rectal absorption in the desert locust, *Schistocerca gregaria* Forskal. III. The nature of the excretory process. A copy of the paper can be obtained from http://jeb.biologists.org/cgi/reprint/41/1/69.

The paper featured in this JEB Classics article (Phillips, 1964c) is one of a trio of articles in Volume 41 of *The Journal of Experimental Biology* in which John Phillips examines the issue of active transport in the insect rectum (Phillips, 1964a,b,c). The rectum of insects is a highly aerobically active organ. Active transport, that is the coupling of metabolic energy to the movement of compounds across the epithelium, is vital for osmoregulation and for the retention of ions, water and nutrients. The paper, entitled 'Rectal absorption in the desert locust, *Schistocerca gregaria* Forskal. III. The nature of the excretory process', pulls together findings from the two previous papers in the series to show how insects manage osmoregulation and excretion to thrive in some of the world's most inhospitable environments.

By the 1960s, it had long been known that epithelia were capable of solute and solvent transport against an osmotic gradient, through a process known as active transport. Whereas today, transport can be studied using histology, protein localization, gene expression or immunofluorescence, in the 1960s active transport was approached principally as a thermodynamic issue. While the term was reserved for situations in which a compound was found to move across an epithelium against its concentration gradient, an additional criterion was that other sources of energy for the process, e.g. electrical gradients, pressure gradients and solvent drag, needed to be ruled out. Demonstrations of active transport therefore required rigorous, detailed studies designed to eliminate all other possible explanations of the movement of a compound against its concentration gradient.

At the time of this article, active transport had been extensively investigated in a variety of vertebrate tissues (Curran and Schwarz, 1960; Anderson and Ussing, 1960). In those studies, many examples of active transport of ions and organic nutrients had been revealed, but no examples of the active transport of water had been found (Robinson, 1960). Given the importance of fluid secretion in biological systems, this was a surprising development. None-the-less, the conclusion had been reached that active transport of water did not occur in vertebrates.

The situation in insects was, however, less clear. In particular, the hindguts of terrestrial insects produce very concentrated excreta and in the process appear to be able to move water from the gut lumen into the hemolymph against a substantial osmotic gradient. It was this process that Phillips undertook to investigate in detail. In doing so, he faced two formidable challenges. The first was to isolate the rectum in order to study the transport of solutes and water under replicable, controlled conditions. He did this by surgically isolating the rectum from input from the midgut and Malpighian tubules by ligating the gut just anterior to the rectum. He then flushed out the gut contents with saline or distilled water and replaced the gut contents with solutions of known makeup (Phillips, 1964a). In this manner, he produced an *in vivo* preparation with natural innervation and tracheation that could be studied in a quantitative, replicated manner.

The second challenge was to be able to accurately measure ionic and osmotic parameters in the tiny fluid samples that could be isolated from the rectal lumen. This problem was solved by cannulating the rectal lumen and removing samples at intervals using a micrometer burette. Chloride ion and osmotic concentrations were determined using the microanalytical techniques developed by Ramsay and co-workers (Ramsay and Brown, 1955; Ramsay et al, 1955). Changes in the volume of fluid in the rectum were the most critical and difficult measurements to make. Phillips solved this problem by using radioactively labelled serum albumen, a

large solute that could not cross the rectal epithelium, as a volume marker (Phillips, 1964a). He was thus able to simultaneously measure changes in chloride concentration, total osmotic concentration and fluid flux.

Using these techniques, Phillips demonstrated that active ion transport occurs in the locust rectum (Phillips, 1964b). Simultaneous microelectrode measurements showed that chloride transport set up an electrical gradient that facilitated the movement of sodium and potassium from the lumen into the hemolymph.

The most startling and significant finding was that water could be transported from the rectum to the hemolymph when the rectum was filled with a solution of 800 mOsm xylose, even though the hemolymph remained at a lower concentration of about 400 mOsm (Phillips, 1964a). This result was surprising since the xylose-containing fluid was devoid of ions and xylose had been shown not to cross the rectal wall. The movement of water could therefore not be attributed to a known osmotic gradient in the appropriate direction nor to a coupling to the movement of solute. In addition, careful measurements of the hydrostatic pressure in the rectum demonstrated that the pressures generated (on the order of 0.2 atm) were far lower than those required to offset the osmotic gradient (11.2 atm). Measurement of ionic concentrations in the rectal fluid during absorption also demonstrated that ions were not cycled into the lumen and then resorbed. In short, the locust rectum met fully the criteria for active transport of water.

In his third paper (Phillips, 1964c), Phillips measured ionic and osmotic concentrations and rates of fluid flow in the midgut, Malpighian tubules and rectum of intact animals. By comparing these to the rates of ion and water uptake he had measured in the ligated preparations, he demonstrated that rectal capacity for ion and water transport exceeds the rate at which ions and water enter the rectum in the urine. His results provided a mechanistic explanation for the observation that the fecal pellets of *Schistocerca* are in equilibrium with a very concentrated solution, but essentially devoid of sodium, potassium and chloride ions. Phillips had simultaneously shown how ions and water were retained in the locust under desiccating conditions, and clarified the mechanisms by which highly concentrated feces were produced (Phillips, 1964c).

These papers demonstrated that water was moving thermodynamically 'uphill' in the absence of simultaneous ion uptake or hydrostatic pressure gradients sufficient to explain the rate of water movement or the equilibrium condition. These were the most complete and rigorous studies to date on rectal function in terrestrial insects. They elucidated the physiological mechanisms used by all terrestrial insects for retaining ions and water and concentrating the excreta.

These papers were of great interest to students of fluid transport in epithelia in the early 1960s. One of the major questions at the time was how could insects produce a concentrated urine through water extraction using only one cell type (the rectal pad cell), a process that vertebrates can only achieve in complex organs involving dozens of cell types (e.g. the mammalian kidney). The results galvanized insect physiologists due to the possibility that insects might possess processes for transporting water that were distinct from those of other animal groups. The papers spawned intense study of insect recta using both ultrastructural (Wall, 1971) and physiological (Phillips, 1970) methods. This resulted in our current understanding that although water does move from a concentrated to a less concentrated fluid in the insect rectum, the water is in fact moved passively by osmotic forces, with intracellular clefts providing the site of hyperosmotic fluid accumulation. These studies in turn contributed to our understanding of the processes by which insect recta are able to take up water from subsaturated atmospheres (Noble-Nesbitt, 1978), a vital process for insects inhabiting extremely dry habitats. As such, the papers by Phillips (Phillips, 1964a,b,c) opened the modern era of rigorous physiological study of insect recta, providing insights into a major adaptation permitting insects to exploit the terrestrial realm.

10.1242/jeb.009589

Timothy J. Bradley
University of California at Irvine
tbradley@uci.edu

References

Anderson, B. and Ussing, H. H. (1960). Active transport. In *Comparative Biochemistry* (ed. M. Florkin and H. S. Mason), pp. 371-402. London: Academic Press.
Curran, P. F. and Schwartz, G. G. (1960). Sodium, chloride and water transport by rat colon. *J. Gen. Physiol.* **43**, 555-572.
Noble-Nesbitt, J. (1978). Absorption of water vapor by *Thermobia domestica* and other insects. In: *Comparative Physiology: Water, Ions and Fluid Mechanics* (ed. K. Schmidt-Nielsen, L. Bolis and S. H. P. Maddrell), pp. 53-66. Cambridge, UK: Cambridge University Press.
Phillips, J. E. (1964a). Rectal absorption in the desert locust, *Schistocerca gregaria* Forskal. I. Water. *J. Exp. Biol.* **41**, 15-38.
Phillips, J. E. (1964b). Rectal absorption in the desert locust, *Schistocerca gregaria* Forskal. II. Sodium, potassium and chloride. *J. Exp. Biol.* **41**, 39-67.
Phillips, J. E. (1964c). Rectal absorption in the desert locust, *Schistocerca gregaria* Forskal. III. The nature of the excretory process. *J. Exp. Biol.* **41**, 69-80.
Phillips, J. E. (1970). Apparent active water transport by insects excretory systems. *Amer. Zool.* **10**, 413-436.
Ramsay, J. A. and Brown, R. H. J. (1955). Simplified apparatus and procedure for freezing-point determinations upon small volumes of fluid. *J. Sci. Instrum.* **32**, 372-375.
Ramsay, J. A., Brown, R. H. J. and Croghan, P. C. (1955). Electrometric titration of chloride in small volumes. *J. Exp. Biol.* **32**, 822-829.
Robinson, J. R. (1960). Metabolism of intracellular water. *Physiol. Rev.* **40**, 112-149.
Wall, B. J. (1971). Local osmotic gradients in the rectal pads of an insect. *Fed. Proc.* **30**, 42-48.