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MOLECULAR SIEVING OF HYDROPHILIC MOLECULES BY THE RECTAL INTIMA OF THE DESERT LOCUST (*SCHISTOCERCA GREGARIA*)*

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INTRODUCTION

Osmotic and ionic regulation in the desert locust (*Schistocerca gregaria* Forskål), as in most insects which have been studied (reviewed by Shaw & Stobbart, 1963; Stobbart & Shaw, 1964), is ultimately achieved by selective reabsorption in the rectum (Phillips, 1961; 1964*a–c*, 1965). The reabsorptive processes include active uptake of chloride, potassium and sodium ions and the ability to produce hypertonic excreta by absorption of water against an increasing osmotic gradient in the absence of net solute movement. While ultrastructural studies (Noirot & Noirot-Thimothée, 1960, 1966; Baccetti, 1962; Baccetti, Mazzi & Massimello, 1963; Phillips, 1965; Irvine, 1966; Gupta & Berridge, 1966*a*, *b*; Berridge & Gupta, 1967; Hopkins, 1966) point to the rectal pad epithelium of insects as the site of active transport, molecules must first penetrate the chitinous intima which lines the rectum before contact is made with these cells.

The rectal cuticle obviously provides protection against mechanical damage to the epithelial cells by faecal material. However, except for the general observation that absorbed substances (i.e. water and small monovalent ions) must penetrate this membrane (Abbot, 1926; Ramsay, 1953, 1955; Phillips, 1964*a–c*), the properties of the rectal intima and the role which the latter might play in the excretory process have not been investigated. It is not clear to what extent the rate and selectivity of reabsorption in the rectum can be attributed to the cuticular barrier. For example, is the impermeability of the rectal wall to certain large molecules such as amaranth, inulin and albumin (Phillips, 1964*a–c*; Irvine, 1966) due to the intima or the epithelium.

In this paper the permeability of the isolated intima to a series of hydrophilic, non-ionic molecules of graded molecular size is reported. In addition some other observations on the physical properties of this membrane are discussed and a comparison is made between the cuticles of the rectum and the integument.

METHODS

Mature adult male *Schistocerca gregaria* which were 3–4 weeks past their final moult and maintained at 28° C. and a relative humidity of 60% on a diet of bran, lettuce and grass were used in all experiments.

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To measure permeability, the intima was prepared as a cannulated sac as follows: With the aid of a micromanipulator, a length of polyethylene tubing (size P.E. 90) was inserted through the anus 1–2 mm. into the rectum and sealed into place with a mixture of beeswax and resin. The integument between the last two abdominal segments was cut circumferentially and the gut partially withdrawn with the aid of the micromanipulator. Tracheae and extraneous tissue were cut away from the rectum. Two loose ligatures of silk or human hair were tied around the hind gut and the ileum was severed. The rectal contents were washed out through the cut end of the ileum by saline injected through the anus. The two ligatures were then pulled tight at the anterior end of the rectal pads.

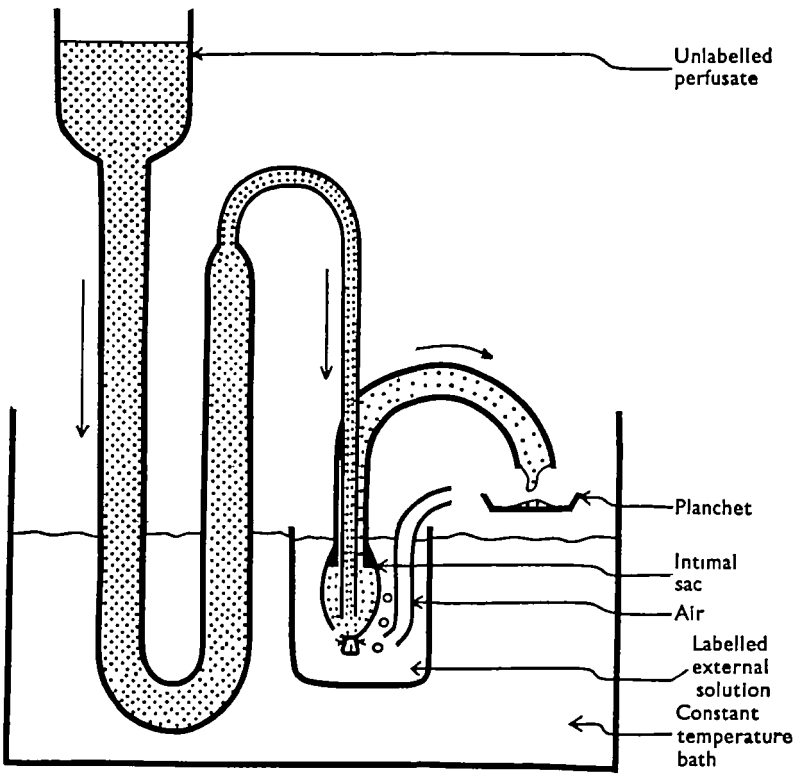

Fig. 1. Experimental arrangement for continuous perfusion of isolated intimal sacs at constant temperature. Permeability is determined by placing isotopes of test molecules in the external solution and measuring the radioactivity and volume of collected perfusate.

In initial experiments the outer layers of tissue were stipped off the cannulated sac, bathed in locust Ringer (Hoyle, 1953), using needle-pointed forceps. An alternative and more convenient method, more frequently used in this study, consisted of filling the cannulated rectum with a saturated solution of the dye amaranth and placing it in tap water for several hours. Most of the tissue fell away from the cuticle under this treatment or could be easily pulled off, leaving the transparent intima alone. The preparations were discarded if traces of dye appeared in the external solution over a period of 12 hr. In such cases gross damage to the intima could usually be observed.

Preliminary experiments indicated that the permeabilities of the intima to water, potassium and sucrose were not significantly different for the two methods of preparation; hence it was concluded that treatment with tap water did not irreversibly alter the properties of the cuticle. This method of preparation is comparable to that used in studies of the integumentary cuticle (Richards, Clausen & Smith, 1953; Beament, 1964, 1965). Evidence that isolated integumentary cuticle exposed to this type of treatment exhibits similar properties to those *in vivo* are mentioned by these authors. Permeability properties of intimal preparations remained unchanged in most cases for several days. The intima was checked daily with amaranth for possible damage. Experiments were carried out within 1–3 days of preparation.

Permeability was measured by observing the rate of radioisotope flux across perfused intimal sacs in the absence of a concentration gradient. Continuous perfusion was achieved by threading small diameter polyethylene tubing (size P.E. 10) through the larger diameter polyethylene cannula to the bottom of the intimal sac (Fig. 1). The tubing was connected to a reservoir of fluid, the height of which could be varied to adjust the rate of perfusion (2–10 ml./hr.). Evidence will be presented in another paper that small hydrostatic pressure gradients (4–12 cm. H_2O) thus established across the intima do not cause a measurable change in the flux of tritiated water across the membrane (Phillips, 1968).

The external solution and the perfusate were of identical composition:

NaCl	5 mM/l.	McIlvaine's phosphate-citrate buffer	20 mM/l.
KCl	5 mM/l.		
$CaCl_2$	5 mM/l.	pH	5·5
$MgCl_2$	5 mM/l.	Test molecule	0·1–1000 mM/l.

The ionic concentrations and pH of this basic solution approximate to those normally observed in the rectal contents of starved locusts supplied with tap water (Phillips, 1964*b*, *c*). Very high osmotic pressures due to high concentrations of organic molecules are also normal for the rectum (Phillips, 1964*b*, *c*). Polyethylene vials containing the external solution were maintained at 28 ± 0·1° C.

^{14}C-labelled or ^{3}H-labelled molecules were added to the external solution in quantities insufficient to alter significantly the total concentration of the substance in question. Perfusate flowing from the larger polyethylene cannula was collected for estimation of radioactivity and volume (by weighing). The rate of perfusion was sufficiently high to maintain radioactivity of the collected perfusate below 2% of that in the external solution; hence, back diffusion of the isotope was negligible. Under these conditions flux of test molecule was calculated by the following equation (modified from Shaw, 1955):

$$f = \frac{A_p VK}{A_e t} \tag{1}$$

where f is the flux in moles/unit time, A_e the radioactivity per unit volume of external medium, and A_p is the radioactivity per unit volume of collected perfusate, V is the total volume flow of perfusate in time t and K is the concentration of test molecule.

The flux rates so obtained were used to calculate intima permeability according to the permeability equation derived from Fick's law (Davson, 1964):

$$f = PAC \tag{2}$$

where f is the flux calculated by equation (1), A the area of the membrane, C the concentration of test molecule and P is the permeability coefficient.

The permeability of individual membranes to several molecules of widely different size was determined in random order. Three to six successive replicate collections of perfusate were made for each test substance and the activities of these were averaged to obtain the permeability values for individual membranes.

Tritiated water (New England Nuclear Corp.) was measured with a 'Nuclear Chicago Mark I' liquid-scintillation counter using Bray's scintillation fluid (Bray, 1960) and the channels–ratio method for quench correction. ^{14}C-labelled amides and sugars (New England Nuclear Corp.) were estimated by the above method or with a 'Nuclear Chicago' thin-window, automatic planchet counter. In all experiments with molecules smaller than raffinose, permeabilities were estimated from a total count of at least 10,000 on samples with activities at least 20 times background. Deuterium oxide was measured, following distillation of samples, by the falling-drop method (Sacks, 1956). The standard deviation for a series of replicate samples was less than $\pm 3\%$ for all of the radioisotope methods and $\pm 6\%$ for D_2O determinations.

Another method of estimating permeabilities (by net diffusion) was used in early experiments with sugars and dyes. A solution of test molecule (0·1–0·5 M) was used as the external medium and a solution of a second solute was used as perfusate. The concentration of the second solute was adjusted empirically to minimize net movement of water. Permeability was calculated from the volume of perfusate collected per unit time and the concentration of test molecule in the perfusate, using modified forms of equations (1) and (2). The concentration of test molecules in the perfusate was below 2% of that in the external medium so that the change in concentration gradient was negligible during these experiments. Sugars were estimated by the anthrone method (Dimler, Scheefer, Wise & Rist, 1952), dyes directly with a spectrophotometer.

It was not possible to determine exact permeability values for larger molecules, which did not penetrate the intima in measurable amounts. In such instances a maximum permeability estimate was calculated using the period of perfusate collection, t, and a radioactivity of test molecule, A_p, which was just detectable by the method employed.

RESULTS

There was considerable variability (3 to 10-fold) in the mean penetration rate of the same test molecule across different preparations. The standard deviation for a series of four to six replicate determinations on a single membrane, however, was relatively small. For example, the standard deviations for replicate measurements of ^{3}HHO flux across individual preparations (21) averaged $\pm 14\%$ (range ± 5–28%) of the mean. The large differences between preparations therefore reflect true permeability differences rather than experimental error. Richards *et al.* (1953) observed a similar degree of variability for preparations of integumentary cuticle.

The average permeability of intimal preparations at 28° C. to 14 hydrophilic mole-

cules of graded molecular size is shown in Table 1. There is a drastic and orderly reduction in the rate of penetration of test molecules with increasing molecular size. Individual preparations all exhibited this relationship. As an approximation, the permeability decreases one order of magnitude for every 1 Å. increase in hydrated radius of the test molecules. Thus, while small molecules such as urea penetrate rapidly, the intima is virtually impermeable to molecules with a radius of 5–6 Å., corresponding to an organic molecule (e.g. disaccharide) of molecular weight greater than 400.

Table 1. *The permeability of the rectal intima at 28° C. to uncharged hydrophilic molecules of graded molecular size*

Molecule	Equivalent hydrated molecular radius (Å.)	Permeability coefficient ($P \times 10^6$ cm.sec.$^{-1}$, mean ± S.E.)	Relative restricted pore area (T_2O = 100)	Concentration of test molecule (molarity)	Numbers of preparations (observations)
Water (D_2O)	1·5 (*a*)	106 ± 31	—	5·5	4 (18)
Water (T_2O)	1·5 (*a*)	77 ± 8	100	< 0·0001	21 (85)
Urea	2·03 (*b*)	12 ± 4	29	0·1	6 (18)
Urea	2·03 (*b*)	18 ± 5	43	0·01	6 (18)
Thiourea	2·18 (*b*)	23 ± 1	55	0·01	4 (16)
Acetamide	2·27 (*b*)	28 ± 5	65	0·1	8 (18)
Acetamide	2·27 (*b*)	26 ± 6	61	0·01	4 (14)
Malonamide	2·57 (*b*)	11 ± 2	27	0·1	6 (19)
Ribose	3·6 (*c*)	8 ± 3	27	0·01	6 (20)
Glucose	4·2 (*c*)	1·1 ± 0·3	5·2	1·0	6 (18)
Glucose	4·2 (*c*)	1·5 ± 0·4	6·5	0·01	3 (12)
Sucrose	5·2 (*c*)	0·092 ± 0·02	0·60	0·01	6 (25)
Trehalose*	5·2 (*f*)	0·042 ± 0·01	0·26	0·5	5 (20)
Raffinose*	6·1 (*c*)	0·017 ± 0·004	0·12	0·25	7 (15)
Amaranth*	7·0 (*e*)	< 0·008	0·05	0·01	24 (24)
Inulin	12 (*d*)	< 0·01	—	0·001	6 (6)
Serum Albumin	37 (*d*)	< 0·01	—	0·00001	8 (8)

* Indicates permeability measured by net diffusion. All other permeability values determined by isotopic flux. (*a*) Wang (1951); (*b*) Goldstein & Solomon (1960); (*c*) Schultz & Solomon (1961); (*d*) Durbin (1961); (*e*) Gordon & Chambers (1941); (*f*) radius of trehalose is assumed to be the same as that for sucrose.

Zeirler (1961) has pointed out that at very high concentrations the relationship between flux rate and concentration may become asymptotic if single-file diffusion occurs. To test whether such departure from linearity was occurring over the range of concentrations used in these experiments, the flux of three molecules was measured at different concentrations, varying by one to two orders of magnitude (Table 1). In no case was the permeability value for a test molecule significantly different at different concentrations; hence any asymptotic trend is not discernible over the range of concentrations used.

Assuming that the test molecules might move through water-filled pores in the intima (see Discussion), the variation in permeability values for different test molecules could be attributed in part to differences in free diffusion rate of these molecules in water. This was taken into account by dividing the membrane permeability coefficient by the free diffusion coefficient for each molecule in water at 28° C. to yield the restricted pore area per unit path length (Pappenheimer, 1953; Paganelli & Solomon, 1957). In Table 1 these values are expressed as a percentage of the restricted

pore area per unit path length for tritiated water. In essence the relative restricted pore area indicates the minimum restriction to molecular penetration which can be attributed to the membrane alone. Dainty (1963, 1965) has pointed out the importance of unstirred layers in considerations of membrane permeability. Since the thickness of these layers can be considered the same for all test molecules under constant experimental conditions, this factor is eliminated when considering relative restricted pore areas.

DISCUSSION

A drastic reduction in permeability with increase in molecular size has been interpreted by many workers as evidence for water-filled pores in membranes (e.g. Davson & Danielli, 1952; Solomon, 1961). Due to steric hindrance at the entrance of pores and viscosity effects in the pores themselves, the diffusion of molecules through pores of relatively uniform size becomes progressively restricted as the dimension of the particles approaches that of the channel (reviewed by Pappenheimer, 1953; Renkin, 1954). Renkin (1954) has demonstrated that his equations to describe this relationship are valid for the diffusion of molecules with effective radii of 2–30 Å. through cellulose membranes 0·005–0·007 cm. thick with effective pore radii of 15–200 Å. These equations have been widely applied to living membranes with much smaller estimated pore radii (reviewed by Solomon, 1961). The Renkin equation to describe diffusion in the absence of solvent flow is given below

$$\frac{A}{A_0} = \left[1-\frac{a}{r}\right]^2\left[1-2{\cdot}104\left(\frac{a}{r}\right)+2{\cdot}09\left(\frac{a}{r}\right)^3-0{\cdot}95\left(\frac{a}{r}\right)^5\right],$$

where A is the restricted pore area per unit path length available for diffusion of molecules with effective hydrated radii a, A_0 is the true pore area per unit path length, and r is the effective pore radius.

The restricted pore areas predicted by the Renkin equation assuming pore radii of 5, 6·5 and 8 Å. are compared in Fig. 2 with experimentally determined values for the rectal intima (Table 1). The restriction to diffusion of hydrophilic molecules across the intima can be accounted for, according to the Renkin equation, by assuming a relatively uniform population of pores having an effective radius of 6·5 Å. A degree of uncertainty regarding the best value is introduced by the difficulty of assigning exact hydrated radii to small molecules (Schultz & Solomon, 1961). The relative size of the test molecules as determined by different methods is similar but absolute values vary considerably, especially for molecules less than 4 Å. in radius. The values used in Fig. 2 for small molecules are those given by Goldstein & Solomon (1960). Allowing for inaccuracy in assigning exact radii to small molecules, the data for the intima fit a pore size of between 6 and 8 Å. A similar relationship has been observed for a series of inorganic ions (Phillips, 1968). Paganelli & Solomon (1957) point out that in spite of the questionable validity of several assumptions made in estimating restricted pore areas and the structural reality of geometrically simplified pores, the result is a convenient model which provides a consistent description of permeability characteristics.

Other observations are in agreement with the above conclusion. No molecule with a radius greater than 6–7 Å. has been observed to penetrate the cuticular intima *in vitro* (Table 1) or the rectal wall *in vivo* (Phillips, 1961, 1964*a*; Irvine, 1966). The list

of such molecules includes serum albumin, inulin, amaranth, light green and raffinose. Secondly, the permeability of the intima to water as estimated by net flow under hydrostatic or osmotic pressure gradients is approximately 50 times greater than that estimated by isotopic flux of tritiated water in the absence of an activity gradient (Phillips, 1961, 1968). This discrepancy, which is almost universally observed for biological membranes (e.g. Prescott & Zeuthen, 1953; Villegas, Barton & Solomon,

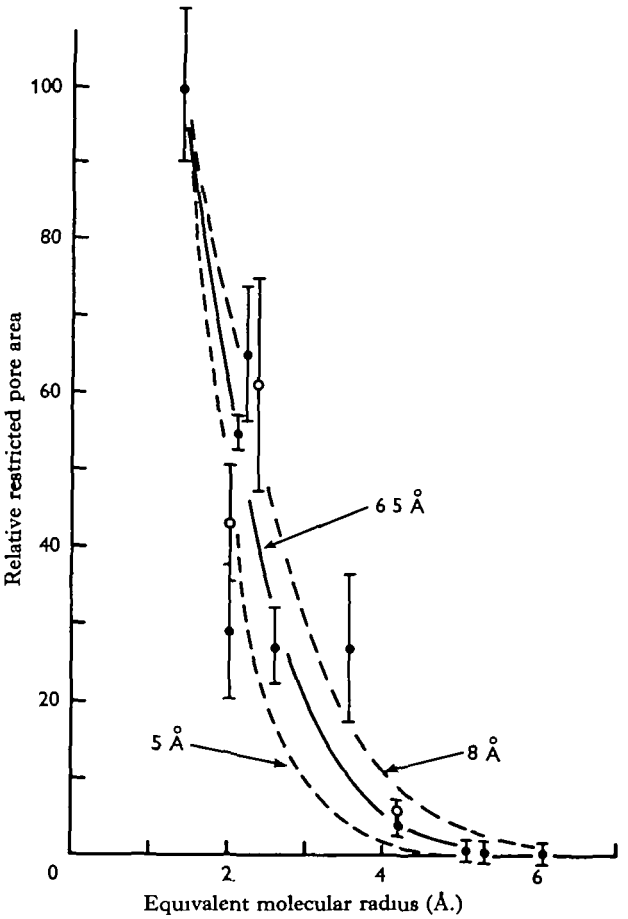

Fig. 2. The relationship between the relative restricted pore area (T_2O = 100%) for diffusion of water-soluble molecules across the cuticular intima and the equivalent hydrated radii of test molecules. The experimental values are compared with the relationship predicted by the Renkin equation for effective pore radii of 5, 6·5 and 8 Å. (broken and solid lines). Vertical lines indicate standard errors. Where restricted pore area was estimated at two different concentrations of a single test molecule, one of the values is indicated by an open circle.

1958), has been presented as evidence for laminar flow of water through pores and used to calculate an independent value for pore radius (e.g. Paganelli & Solomon, 1957). Using the method of these authors, an independent value for pore radius of 20–24 Å. has been calculated for the rectal intima of the locust (Phillips, 1968). Considering the multilaminar structure of the intima and the assumptions made in this method, a value of 20–24 Å. represents an upper limit only (Solomon, 1961) and

cannot be considered inconsistent with the pore size suggested for this membrane by the Renkin equation.

Beament (1964, 1965) has pointed out that three types of insect epithelium (rectum, integument and tracheole) which are capable of solvent transport in the absence of net solute movement all possess a chitinous cuticle. He suggests that the capacity to move water against a gradient is related to the properties of the cuticle, which acts as a valve. It is of interest therefore to compare as far as possible the properties of the intima, which has not received previous attention, with the integumentary cuticle, which has been extensively studied (reviewed by Beament, 1961*a*, 1964, 1965; Richards, 1951; Ebeling, 1964).

Table 2. *Relationship between permeability of the rectal intima of the desert locust and lipid solubility of molecules* (r)

(Molecules are arranged in increasing order of lipid solubility)

Molecule	Molecular weight (M)	Permeability ($P \times 10^6$ cm.sec.$^{-1}$)	Olive oil: water partition coeff. ($r \times 10^3$)*	$PM^{\frac{1}{2}}$ ($\times 10^6$)
Water (T_2O)	18	77	<0·00	450
Ribose	150	8	0·03	98
Glucose	180	1	0·03	16
Sucrose	342	0·1	0 03	2
Malonamide	102	11	0·08	111
Urea	61	15	0·15	116
Acetamide	59	27	1·11	208
Thiourea	76	23	1·37	201

* Values from Davson & Danielli (1952); Goldstein & Solomon (1960).

The permeability of cuticle from the abdominal tergum of the desert locust is directly related to lipid solubility of molecules and this relationship can be attributed to a wax layer (Treherne, 1957). The presence of a wax layer on the lumen surface of the intima might be inferred from the homology of this membrane to that of the integument as well as from observations on wetting properties. The rectal cuticle of the desert locust (J. E. Phillips, unpublished observations) and *Rhodnius* (Maddrell, 1963) are both hydrophobic on the lumen side. The possibility of a relationship between lipid solubility and permeability of rectal cuticle is considered in Table 2. No direct correlation is evident between lipid solubility of test molecules as indicated by their olive oil: water partition coefficients, r, and intima permeability corrected for molecular weight, $PM^{\frac{1}{2}}$. However, molecules of high lipid solubility were not used in this study so that the possibility of enhanced penetration of such molecules cannot be excluded. At least for compounds of low lipid solubility permeability of the intima is determined largely by the hydrated radius of molecules.

Permeabilities of rectal and integumentary cuticles to selected molecules are compared in Table 3. The rectal intima is two to three orders of magnitude more permeable to urea, thiourea and water than is the integumentary cuticle. After removal of the wax layer of the integumentary cuticle by chloroform extraction or by abrasion this difference is reduced to one order of magnitude; moreover, permeability of the latter cuticle following this treatment is directly related to molecular size rather than lipid solubility of test molecules. Treherne (1957) concludes that following wax

removal '...diffusion is similar to that through relatively large liquid-filled spaces'. As a working hypothesis, therefore, it is suggested that the essential difference between the two types of cuticle is the absence of a complete (i.e. continuous) wax layer over the surface of rectal cuticle.

Beament (1961 *b*) has demonstrated the existence of an electrostatic potential difference of 200 mV. across the integumentary cuticle of the cockroach. This potential appears to be associated with a continuous, orientated monolayer of lipid molecules. Electrical potential differences were measured, by a method previously described (Phillips, 1964*b*), across the isolated rectal intima of the desert locust, bathed on both sides with buffered KCl solutions (basic buffered ionic media described in methods

Table 3. *A comparison of some permeability values for cuticles from the integument and rectum*

Type of cuticle	Permeability coefficient ($P \times 10^6$ cm. sec.$^{-1}$) Urea	Thiourea	Water (isotope flux)
1. Intima of *Schistocerca gregaria*	15	23	77
2. Abdominal tergum of *Schistocerca gregaria**	0·02	0·07	—
3. As (2) with wax layer removed*	1·7	1·9	—
4. Integumentary cuticle of *Sarcophaga bullata*†			0·78

* Treherne (1957). †Richards *et al.* (1953).

Table 4. *Electrical potential difference at 28° C. across isolated intimal membranes bathed on both sides with buffered ionic solutions of identical composition.*

(Sign refers to haemocoel side. The normal pH of rectal fluid is 5·5)

pH of solution	Electropotential difference in mV. (mean ± S.E., no. of observations) 0·01 M-KCl	1·0 M-KCl
3·0	+0·02 ± 0·1 (13)	−0·1 ± 0·05 (11)
5·5	+0·2 ± 0·3 (12)	−0·04 ± 0·07 (11)
7·8	−0·6 ± 0·2 (7)	−0·08 ± 0·13 (9)

with added KCl) of identical concentrations (Table 4). Unlike the integumentary cuticle of the cockroach, the rectal intima shows no electropotential difference over a pH range from 3·0 to 7·8. This observation is consistent with the hypothesis that lipid does not form a complete monolayer over the surface of the rectal intima, since any electrostatic potential associated with a discontinuous lipid monolayer might be short-circuited via water-filled pores.

There is ultrastructural evidence for pores (i.e. wax canals) of 30–60 Å. radius, containing lipid in the middle phase configuration, in the epicuticle of the integument (Locke, 1965). Assuming that similar-sized pores exist in rectal cuticle and allowing for an orientated monolayer of wax absorbed on the walls of such pores, wax canals

might provide water-filled channels of a magnitude similar to those postulated in this paper. Excess lipid might be removed by continual passage of material through the rectum so that the wax of the epicuticle surface and wax canals is reduced to a strongly adsorbed monolayer. Alternately, the passage of material through the rectum might cause abrasion of a continuous wax layer.

Turning to a consideration of the part which the rectal intima plays in the excretory process in the locust, these experiments indicate a hitherto unsuspected function. This membrane acts as a molecular sieve which allows rapid exchange of water, salts (Phillips, 1961, 1968), and other small molecules (e.g. monosaccharides and probably amino acids) between the lumen and the epithelium. These substances probably enter the lumen of the Malpighian tubules by diffusion following the active secretion of potassium and to some extent water and sodium (Ramsay, 1958). They include basic metabolites and substances most important in osmotic and ionic regulation. Control of their reabsorption probably resides in the epithelial layer (Phillips, 1964*a–c*).

At the other extreme are large organic molecules which accumulate in the rectum as fluid circulates through the Malpighian tube-rectal system due to the impermeability of the intima. Many large molecules, such as amaranth (Phillips, 1961), phenol red (Ramsay, 1954), fluorescein (Gersch, 1942), and many other dyes (Lison, 1942) are activity secreted by the Malpighian tubes. While these substances are not commonly found in insects, compounds with similar molecular configurations may normally appear in the haemolymph as a result of ingestion, autolysis, or metabolic activities. The ability of the Malpighian tubes to secrete dyes could indicate the presence of one or more carrier systems which can actively transport a large number of organic compounds having a similar basic structure. This has been shown for vertebrate tubules (Wilbrandt, 1954). Such transport mechanisms might have evolved in insects as a consequence, first, of the toxicity or pharmacological activity which many organic molecules exhibit at very low concentrations, and secondly, of their large size which precludes their rapid removal by diffusion into the lumen of Malpighian tubules.

Koch (1954), for example, found that very low concentrations of acidic dyes inhibited cholinesterase activity and the active transport of sodium chloride across the gills of *Eriocheir* and the anal papillae of *Chironomus* larvae. The intima lining the rectum would allow accumulation of such substances in the rectum while protecting the epithelium from their detrimental effects, including inhibition of active transport.

Uric acid, being the main form in which nitrogen is excreted by the locust, requires special mention. The intima might be expected to restrict considerably (but alone not to prevent) reabsorption of this substance from the rectum. However, rapid water absorption and restricted urate movement across the intima should lead to precipitation of most of the urate in the lumen. This might be enhanced by the acidification of the rectal fluid (Phillips, 1961) for uric acid is less than one-twentieth as soluble as sodium or potassium urate. Finally, the electrical potential difference across the rectal wall (Phillips, 1964*b*) opposes the reabsorption of any urate remaining in solution.

SUMMARY

1. The permeability of perfused intimal sacs to fourteen non-ionic, hydrophilic molecules of graded molecular size was estimated by radioisotope flux.

2. The rectal cuticle acts as a molecular sieve severely restricting the rate of penetration of molecules with increasing hydrated size.

3. The penetration of test molecules was as predicted by the Renkin equation for a uniform population of water-filled pores having radii of 6·5 Å.

4. The properties of cuticles from the rectum and the integument are compared and the role of the rectal intima in the excretory process in the desert locust is discussed.

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