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THE PERMEABILITY OF THE CUTICULAR LINING OF THE INSECT ALIMENTARY CANAL

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SUMMARY

1. The permeability of the cuticle lining the foregut and hindgut of adults of *Schistocerca gregaria*, *Gromphadorhina portentosa*, and *Leucophaea maderae*, and larvae of *Manduca sexta* was investigated.

2. In *Schistocerca*, *Manduca*, *Leucophaea*, and *Gromphadorhina* the cuticle lining the foregut has a very low permeability, while that lining the ileum (*Schistocerca*, *Manduca*, *Leucophaea*, and *Gromphadorhina*) and the rectum (*Schistocerca* and *Manduca*) is much more permeable.

3. The low permeability of cuticle lining the crop in the insects examined is suited to the crop's function as a temporary store of ingested material.

4. In *Schistocerca gregaria*, the cuticular lining of the ileum and rectum were found to be equally permeable. Each allows relatively rapid passage of small neutrally charged hydrophilic molecules. Both, however, showed a reduced permeability to anions and an enhanced permeability to cations. The cuticular lining of the hindgut would thus not be a barrier to the reabsorption of useful substances known to appear in the fluid reaching the hindgut from the Malpighian tubules, but it would, to some extent, retain in the lumen the relatively large anions actively excreted by the Malpighian tubules.

5. The cuticle lining the colon of *Schistocerca gregaria* has a much lower permeability than has that lining the rest of the hindgut. This accords with the idea that the colon is thought not to be involved in absorption.

INTRODUCTION

The layer of cuticle which lines the foregut and hindgut of insects has the obvious function of mechanical protection for the underlying epithelial cells. This is of particular importance in herbivorous insects. However it is also important that it should have permeability properties appropriate to the function of the different regions of the gut that it lines. Phillips & Dockrill (1968) showed that the cuticular intima of the rectum of *Schistocerca gregaria* selectively restricts permeation; only small molecules penetrate it with any ease. The lining was more or less impermeable to molecules with a radius of more than 0·5–0·6 nm. Such a restricted permeability is important because the rectum reabsorbs virtually all the water from the contents of the lumen. This leads to a high concentration of substances in the remaining fluid. Some of these substances are toxic and at high concentrations they would interfere

with the action of the rectal cells, on whose reabsorptive abilities the well-being of the insect vitally depends. The rectal cuticle denies such toxins access to the rectal epithelium.

It has been supposed that the mode of operation of the insect excretory system is based on non-selective collecting of substances into a primary excretory fluid by the Malpighian tubules followed by reabsorption of useful molecules in the rectum. If this were the case, then difficulties would arise if the Malpighian tubules were more permeable to dissolved substances than is the rectum, as some useful substances committed to the primary excretory fluid might not then be recoverable. In the

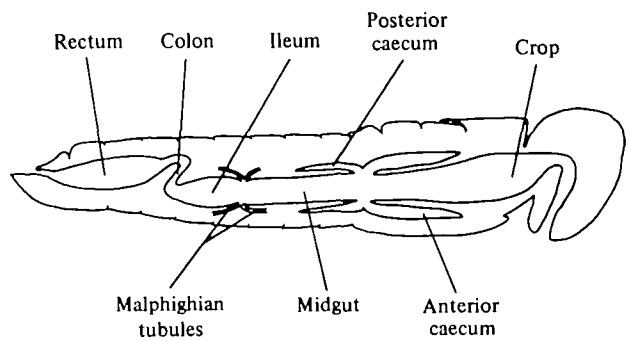

Fig. 1. Diagrammatic representation of a sagittal section of *Schistocerca gregaria* to show the disposition of the various parts of the alimentary canal.

locust *Schistocerca gregaria*, it seems that such a mismatching does occur. Maddrell & Gardiner (1974) showed that the Malpighian tubules of this insect are moderately permeable to such compounds as the trisaccharide maltotriose (MW 504) and inulin (MW 5200), whereas Phillips & Dockrill found the rectal intima to be virtually impermeable to substances of molecular weight greater than 400. However, it must be remembered that the fluid derived from the Malpighian tubules passes through the anterior hindgut before entering the rectum (Fig. 1). Perhaps absorption of relatively large substances might occur there. In the present paper, we have investigated the cuticular lining of the walls of the hindgut anterior to the rectum to see if the permeability is higher than that of the rectum. For comparative purposes we have also examined the permeability properties of the cuticle lining the foregut (crop) and the cuticular intima of the colon, a short region of the hindgut between the main length of the anterior hindgut, the ileum, and the rectum (see Fig. 1). In addition we report on the permeability of the gut cuticle of adults of two species of cockroach, *Gromphadorhina portentosa* and *Leucophaea maderae* and of the larvae of a Lepidopteran, *Manduca sexta*.

MATERIALS AND METHODS

Insect material

Adult *Schistocerca gregaria* Forskal of average weight 2·2 g were taken 1–2 weeks after their last moult from a laboratory culture maintained on a diet of cabbage and rabbit pellets. Laboratory-reared adult *Gromphadorhina portentosa* Schaum and *Leucophaea maderae* Burmeister, and 5th instar larvae of *Manduca sexta* Johannson were also used.

Experimental methods

The experimental preparations of cuticle were prepared by removing the entire gut and allowing it to soak overnight in distilled water, after which the cuticular lining could easily be separated from the cells of the gut by peeling the two apart. An alternative pretreatment was to freeze the insects at −20 °C; particularly with the cock-

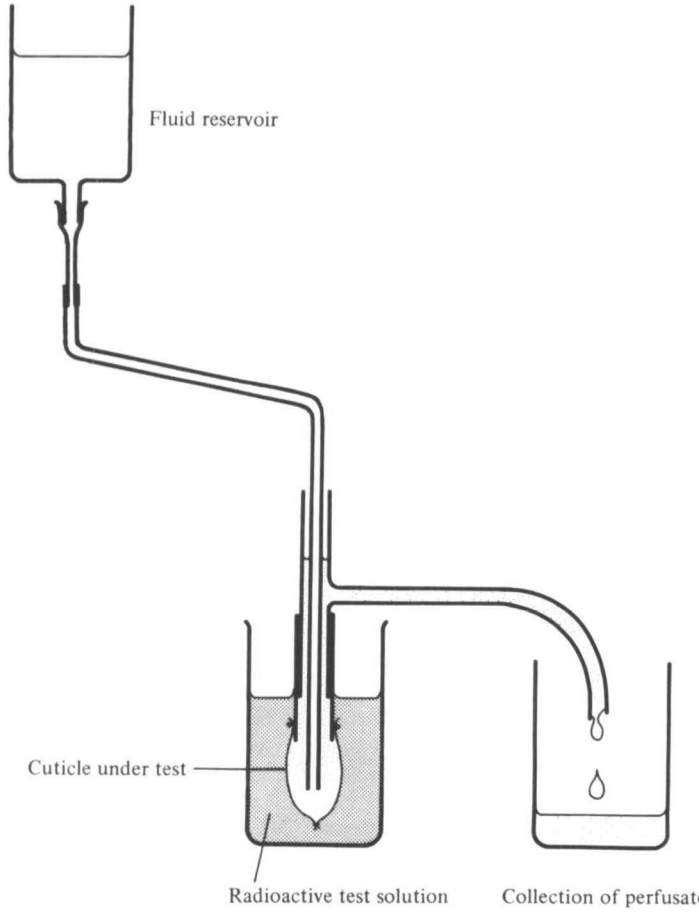

Fig. 2. The experimental arrangement involved in measuring the permeability to different substances of the cuticle lining the gut.

roaches, the subsequent separation of the cuticle from the underlying epithelium was simple. The two techniques were found to give identical results. It was not possible to effect separation in freshly killed material.

The cuticular lining, in the form of a hollow tube, was pulled over a piece of polythene tubing of the appropriate diameter and tied in place with a silk ligature. The free end was then tied off so that the polythene tube carried a closed sac of cuticular material at its end. The other end of the tube was then fitted to a stainless-steel T-piece as is shown in Fig. 2. A second narrower polythene tube attached to a fluid reservoir was then passed down the tube so that its end lay not more than 1 mm above

the bottom of the preparation. Fluid was passed down this tube at a rate which could be controlled by altering the height of the reservoir. In the majority of the experiments this rate was kept at about 180 μl min^{-1}. The preparations were normally kept immersed in distilled water until required. In use, each preparation was first tested for leakage by passing through a solution of amaranth, a deeply coloured red dye (MW 535). Following this, a K-rich saline was perfused through the inner polythene tube. The rapid removal of dye showed that circulation of fluid in the cuticular sac had left no perceptible unstirred regions. A K-rich saline was used as an approximation to the composition of the fluid that the hindgut cuticle might experience *in vivo* in contact with its luminal face. It had the following composition (mM); K^+ 138, Na^+ 14, Ca^{2+} 2, Mg^{2+} 8·5, Cl^- 159, HCO_3^- 10, $H_2PO_4^-$ 4, and glucose 34; the pH of the solution was 6·7. The perfused preparation was then lowered into a small vial with 1–2 cm^3 of saline in it, containing one of a series of radioactive test substances. The bathing saline was iso-osmotic to the K-rich saline and contained (mM); Na^+ 143, K^+ 9, Ca^{2+} 2, Mg^{2+} 8·5, Cl^- 159, HCO_3^- 10, $H_2PO_4^-$ 4, and glucose 34 (pH 6·7). Samples of the effluent perfusate were first taken at about 2 min intervals to find out how long it took for its radioactive content to reach a steady state. In no case did this take more than 6 min. A period of at least 10 min was allowed in all following experiments. After this initial period, samples of the perfusate were taken for determination of the permeability of the cuticular sac to the particular substance under test. Samples were either taken every few minutes, in which case the radioactive content of the entire sample was determined, or the perfused fluid was allowed to collect for, say, 20 min and a 200 μl aliquot was removed for counting. Even with the most permeant substance used the perfusate never contained radioactivity at more than 1% of the concentration in the bathing fluid. Back diffusion of the isotope can thus be ignored.

The permeability of a cuticular sac to a particular test substance was calculated from the equation $P = C/Nat$, where C is the total number of radioactive counts collected in time t (s), N is the concentration of radioactive material in the bathing fluid (cpm cm^{-3}), and a is the area of the cuticular sac (cm^2). Initially, measurement of the area of cuticle in a preparation was carried out in two ways. First the length and diameter of the sac were measured with an ocular micrometer and the area of cuticle calculated assuming the sac to be cylindrical. Next the sac was cut off the polythene tube and the piece of cuticle distal to the bottom ligature was cut off. The sac was then cut longitudinally and laid out as a flat sheet on a measuring grid. Its dimensions were determined, again with an ocular micrometer, and its area determined. The two methods gave such closely similar results that in all later experiments only the first method was used.

The cuticular lining of the crop in *Schistocerca* is so extensive (its area is about 4 cm^2) that measurements were made on only a short section of it taken about half way along its length.

One difficulty we had in our earlier experiments is worth recording. [^{3}H]inulin appeared to cross the cuticular sacs at surprisingly high rates. This was found to be due to trace levels of radiolabelled impurity which did not appear in subsequent chromatographic checks for purity. We believe that tiny amounts of tritium label may have exchanged with hydrogen in the solvent water molecules. With such a slowly permeating substance as inulin, even a few extra counts from such a spurious source

can greatly affect the results. In the experiments described we report only the results obtained with ^{14}C-labelled inulin.

RESULTS

Schistocerca gregaria

Table 1 summarizes our findings on the permeability of the cuticle lining the alimentary canal of adult *Schistocerca*. Included in the table are corresponding figures from the earlier work of Phillips & Dockrill (1968). On the important point of the relative permeabilities of the cuticle lining the anterior hindgut and rectum, our results show that the cuticle lining the anterior hindgut is scarcely more permeable to most of the test molecules than is the cuticle lining the rectum.

Compared with the values determined by Phillips & Dockrill, our results agree in showing only negligible permeability to substances as large as inulin, but we found the rectal cuticle to be somewhat more permeable for most of the other substances investigated. The differences are not large, but we made some attempts to find a factor which might explain them. One possibility was that the locusts we used were 1–2 weeks after their final moult, whereas Phillips & Dockrill used insects some 2 weeks older. To see if this factor could cause differences, we repeated some of our experiments on older locusts, The results (Table 2) showed that there was no significant difference in the permeability of rectal cuticle taken from young or old adult insects. Another possibility was that our experiments were done using a saline of near neutral pH (6·7) and those of Phillips & Dockrill at pH 5·5. We therefore investigated the permeability of rectal cuticle using alternately the same more acid solution used by Phillips & Dockrill and the K-rich near-neutral saline as before. The results are set out in Table 3 and show that acid conditions do somewhat increase the permeability to negatively charged molecules and depress the permeability to substances carrying a net positive charge. However, the permeability to more nearly neutral substances such as glycine, glucose, and inulin is not noticeably changed.

The permeability of the cuticle lining the foregut we found to be very low (Table 1). Interestingly, the permeability of the cuticular lining of the colon was also low, about 15 times lower than the cuticle lining either the ileum, which lies anterior to it, or the rectum, posterior to it,

As Phillips & Dockrill (1968) pointed out, it is advisable to test permeability at more than one concentration of a test substance to rule out the possibility that there might be a non-linear relationship between flux rate and concentration. In the cases of five test molecules and using two concentrations differing by about two orders of magnitude we checked this point on cuticle from colon, foregut and ileum. The results (Table 4) show that in no case was the permeability value for a test molecule significantly different at the different concentrations.

We were surprised to find (Table 1) that the permeability of the cuticle lining the ileum was apparently not noticeably higher than that of the rectal lining. In case a small difference had been lost in the variation due to slight differences in age and/or the physiological state of locust used, we compared the permeability of sacs of cuticle taken from the ileum and rectum of the same insect (Table 5). For most substances, the cuticle lining the ileum did show apparently slightly higher permeabilities, but in

Table 1. *The permeability of the cuticular lining of different regions of the gut of* Schistocerca gregaria *to various substances. In all cases the concentration of the test substance was less than* 1 mM

Permeability coefficients ($P \times 10^6$ cm s^{-1}, mean ± S.E. (n))

Substance	MW	Foregut (crop)	Anterior hindgut (ileum)	Colon	Rectum (this investigation)	Rectum (figures from Phillips & Dockrill, 1968)
Acetate	59		4·74 (2)			
Urea	62	0·059 ± 0·012 (8)	82·81 ± 9·35 (18)	0·45 ± 0·07 (9)	75·06 ± 5·19 (9)	15
Glycine	75	0·016 ± 0·006 (6)	57·59 ± 13·95 (5)	0·81 ± 0·14 (3)	37·25 (1)	
Alanine	89		25·65 ± 10·06 (4)	0·26 (2)	12·59 (2)	
Serine	105	0·0103 ± 0·0014 (4)	40·01 ± 4·25 (5)	0·38 ± 0·09 (8)		
Proline	115		32·86 ± 5·61 (13)	1·22 (1)	21·77 ± 4·06 (9)	
Leucine	131		9·21 ± 2·69 (4)			
Lysine	146	0·0091 ± 0·0521 (4)	12·11 ± 2·03 (4)	0·16 ± 0·09 (4)		
Ribose	152		21·40 ± 3·14 (6)			8
Glucose	180	0·0063 ± 0·0012 (4)	13·90 ± 1·47 (10)	0·06 (2)	16·82 ± 2·07 (6)	1·3
Tyrosine	187		0·44 ± 0·17 (6)		1·17 (1)	
β-methyl glucoside	192		2·18 ± 0·49 (6)		1·63 (1)	
Atropine	289	0·0053 ± 0·0012 (4)	5·82 ± 1·09 (7)			
Sucrose	342	0·004 ± 0·001 (4)	0·50 ± 0·07 (14)	0·061 (1)	0·40 ± 0·04 (7)	0·092
Trehalose	342		0·53 ± 0·14 (6)		0·46 ± 0·04 (6)	0·042
Benzyl-penicillin	372		0·34 ± 0·08 (8)		0·66 (1)	
Ouabain	585	0·022 ± 0·004 (4)	0·96 ± 0·13 (5)		0·25 ± 0·06 (10)	
Inulin	5200		0·012 ± 0·002 (3)		0·014 ± 0·001 (4)	< 0·01

Table 2. *Permeabilities of rectal cuticle taken from adult* S. gregaria *of different ages. Values are expressed as* $P \times 10^6$ *cm* s^{-1}, *mean* ± S.E. ($n = 4$)

Test substance	3–4-day-old insects	28-day-old insects
Urea	80·5 ± 14·8	73·9 ± 7·7
Glucose	12·16 ± 1·96	11·14 ± 1·57

Table 3. *The pH dependence of the permeability of the cuticular lining of the rectum. Values are expressed in* $P \times 10^6$ *cm* s^{-1} (*mean* ± S.E., *n in brackets*).

	pH	
Test substance	5·5	6·7
Urea	149 ± 17 (5)	262 ± 31 (5)
Glycine	30·4 (2)	37·3 ± 6·0 (5)
Glucose	8·4 (2)	9·6 (2)
PAH	0·70 (2)	0·56 (2)
Uric acid	0·34 ± 0·15 (3)	0·049 ± 0·015 (3)
Ca^{2+}	4·13 ± 0·72 (4)	8·30 ± 1·23 (4)
SO_4^{2-}	0·054 ± 0·012 (4)	0·033 ± 0·012 (4)

Table 4. *The permeability of the cuticular lining of regions of the gut of* S. gregaria *to various small molecules at different concentrations*

		Mean value of permeability coefficient of cuticle ($P \times 10^6$ cm s^{-1})		
Substance tested	Concentration (mM)	Foregut	Colon	Ileum
Serine	0·0035		0·43 (0·27, 0·68)	
	2·37		0·45 (0·26, 0·65)	
Urea	0·04	0·088 (0·083, 0·093)	0·31 (0·30, 0·31)	54·5 ± 2·1 ($n = 3$)
	2·37	0·087 (0·085, 0·089)	0·31 (0·28, 0·34)	58·9 ± 7·1 ($n = 4$)
Proline	0·007			47·2 (61·9, 32·5)
	2·18			43·3 (52·8, 33·8)
Tyrosine	0·005			0·32 (0·38, 0·25)
	2·50			0·41 (0·45, 0·37)
Sucrose	0·008			0·52 (0·61, 0·43)
	2·29			0·48 (0·56, 0·40)

Table 5. *Permeability of the cuticular linings of the ileum and rectum from the same individuals of* S. gregaria. *Values are expressed as* $P \times 10^6$ *cm* s^{-1}, *mean* ± S.E. ($n = 7$).

Probability values determined by the paired observation '*t*' test.

Substance	Ileum	Rectum	*P*
Urea	99·70 ± 8·00	80·30 ± 4·62	< 0·025
Proline	27·64 ± 8·35	23·93 ± 4·85	NS
Ribose	21·40 ± 3·14	22·12 ± 2·34	NS
Glucose	17·64 ± 1·14	15·37 ± 1·67	< 0·1
Trehalose	0·53 ± 0·14	0·46 ± 0·04	NS
Sucrose	0·52 ± 0·14	0·37 ± 0·02	NS
Ca^{2+}	52·14 ± 4·10	40·67 ± 3·05	< 0·025
SO_4^{2-}	0·094 ± 0·014	0·082 ± 0·023	NS

NS = not significant.

Table 6. *Permeabilities of cuticle lining the alimentary canal in* Manduca sexta, Leucophaea maderae *and* Gromphadorhina portentosa. *Values are* $P \times 10^6$ *cm* s^{-1}

	Manduca sexta			*Leucophaea maderae*		*Gromphadorhina portentosa*	
Substance	Foregut	Ileum	Rectum	Foregut	Ileum	Foregut	Ileum
Urea	0·30	82·2	73·9 ± 11·6 (4)				
Acetate	—	6·3	—	0·016	49·5	0·002	28·0
Serine	—	13·2	—	—	—	0·12	14·5
Proline	2·6	9·5	16·5 ± 5·3 (4)				
Glycine	—	—	—	0·074	3·38	0·042	
Leucine	—	—	4·6				
Atropine	—	—	—	0·012	2·66	0·018	3·35
Lysine	—	—	—	—	—	0·020	0·93
Ribose	—	5·4	9·2				
Glucose	0·25	4·4	3·2				
Trehalose	—	3·8	1·15				
Sucrose	0·11	1·2	1·7	—	—	0·06	0·19
Ouabain	—	—	—	0·052	0·62	—	0·015
Ca^{2+}	0·63	1·9	0·9				
SO_4^{2-}	0·02	2·1	2·9				

only two cases, those of urea and Ca^{2+} ions, was the difference significant. Evidently, the cuticle lining the ileum and rectum is very similar in its permeability properties.

Manduca sexta, *Leucophaea maderae*, and *Gromphadorhina portentosa*

Table 6 sets out the results of determinations of the permeability of cuticle lining different regions of the foregut and hindgut in these other species. The number of determinations made are not large so that it would be unwise to draw other than general conclusions from the results. Nonetheless it is clear that, in the cockroach species, the lining of the foregut is as little permeable as it is in *Schistocerca*, and that even in *Manduca sexta*, it is considerably less permeable than the cuticle of the hindgut. In *Manduca*, the permeability of the cuticle lining the ileum differs little from that lining the rectum. It is thus similar in this respect to the cuticle lining the hindgut of *Schistocerca*. The cuticle lining the ileum in the two cockroach species appears to be rather less permeable than that of *Manduca* and *Schistocerca*. Finally, the hindgut cuticle in *Manduca* does not show the great difference in permeability to Ca^{2+} and SO_4^{2-} ions that is apparent in *Schistocerca*.

DISCUSSION

The results presented in this paper show, unexpectedly, that in *Schistocerca* the permeability of the cuticle lining the ileum is not significantly more permeable than that lining the rectum. This was surprising because, as emphasized earlier (p. 228), it would appear that useful substances appearing in the primary excretory fluid elaborated by the Malpihigan tubules could not readily be reabsorbed through cuticle of a low permeability. Three factors reduce this difficulty. First, in our experiments the permeability of the cuticle lining the ileum and rectum was significantly higher than Phillips & Dockrill (1968) found for the rectum. A second and more important point is that, as recently shown (Knowles, 1975), some insect Malpighian tubules actively reabsorb sugars such as glucose and trehalose from the lumen. It may well be, therefore, that the concentration of trehalose (the most important carbohydrate constituent of the haemolymph) in the fluid entering the midgut is already very low, so that difficulties of absorption through the cuticle may not be important. Thirdly, the other important organic constituents of the haemolymph are amino acids and lipids. The presence of amino acids in the primary excretory fluid does not cause the same difficulties as do larger molecules. This is because they can readily penetrate the hindgut cuticle and can therefore be reabsorbed from the lumen as, indeed, Balshin & Phillips (1971) have shown does occur. Lipids in circulation, which are of great importance to the locust as the main fuel for flight (Weis-Fogh, 1952), avoid being excreted by being circulated, not as free diglyceride, but in the form of lipoprotein, a conjugate of diglyceride with protein (Gilbert, 1967). Such large molecules would not cross the walls of the Malpighian tubules of the locust at significant rates (Maddrell & Gardiner, 1974).

The colon (Figs. 1, 3) is rather narrower in diameter in *Schistocerca* than is the ileum or rectum and is S-shaped in the dorsoventral direction. Our findings show that its cuticular lining is remarkable in that it is very much less permeable than is the lining of the ileum or rectum. The functions of the colon include that of breaking up

the continuous flow of material from the ileum. To do this, the colon first constricts the cylinder of materials in the lumen and then, by bending, breaks the peritrophic membrane between the separated pieces (Goodhue, 1963). This presumably yields suitable lengths of material which can then be individually processed by the rectum prior to elimination as faecal pellets. After these manoeuvres, the colon contains no solid matter and very little fluid. Presumably, then, reabsorption of useful substances does not occur in the colon and our finding that its cuticle has a greatly reduced permeability is entirely in accord with its known function.

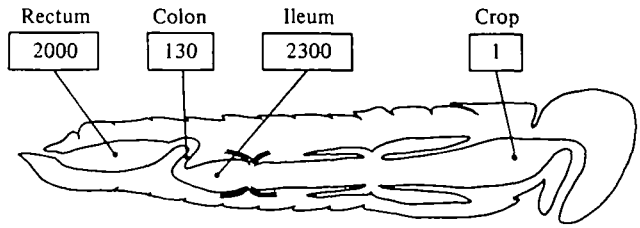

Fig. 3. The relative permeabilities of the cuticle lining the various parts of the alimentary canal of *Schistocerca*.

Our findings on the permeability of the cuticle lining the foregut and hindgut of *Schistocerca* are summarized in Fig. 3.

In the locust *Schistocerca*, the cockroaches *Gromphadorhina* and *Leucophaea*, and in *Manduca* the cuticle lining the crop has a very low permeability. This presumably explains earlier qualitative findings that the crop wall in several insect species has a low permeability (Treherne, 1957, 1958, 1967). Such a low permeability allows this region of the gut to be used as a temporary store for ingested material very different in its osmotic concentration from the haemolymph. The rate at which the crop contents are passed on to the much more permeable midgut is regulated in a manner dependent on the osmotic concentration of the contents (Treherne, 1957). It seems reasonable to propose that this would serve to minimize the effects of osmotic water movements between the haemolymph and the lumen of the midgut.

From his work on the cuticular lining of the rectum of *Schistocerca*, Phillips has concluded that it behaves as if it contained water-filled pores of radius 0·6–0·7 nm lined with negative charges having a pK of about 4 (Phillips, 1977). Our results are consistent with this proposal. For example, we find (Table 1) that the cuticle is almost impermeable to inulin which has an equivalent hydrated molecular radius of about 1·2 nm (Durbin, 1961). In addition, we have found that the cuticle lining the hindgut of *Schistocerca* is very much more permeable to cations than it is to anions (Tables 1, 3, and 5). This suggests that the cuticle carries a preponderance of fixed negative charges. Finally we find that although changing the pH from 6·7 to 5·5 (Table 3) decreases the permeability of the cuticle to cations and increases it to anions, the effects are small, as they would be if the pK of the fixed charges were about 4.

The significance of the differential permeability to anions and cations may lie in the facts that metabolically produced anions are important subjects for excretion in insects (Smith, 1962) and that insect Malpighian tubules possess active transport systems capable of accelerating their removal from the haemolymph (Maddrell *et al.*

1974). In an insect such as *Schistocerca* that concentrates the contents of the hindgut very much (Phillips, 1964), the low permeability of the hindgut cuticle to anions is appropriate in that it reduces the tendency for excreted anions to be returned to the haemolymph.

Larvae of *Manduca* which feed on a diet relatively rich in water do not need to resorb water to the same extent. Substances in the hindgut lumen presumably do not therefore become so concentrated and so a low permeability to anions is not so obviously an advantage. It is perhaps not surprising then that the hindgut cuticle of *Manduca* does not in fact show nearly such a large difference in its permeability to Ca^{2+} and SO_4^{2-} ions as does that of *Schistocerca*.

REFERENCES

Balshin, M. & Phillips, J. E. (1971). Active absorption of amino-acids in the rectum of the desert locust (*Schistocerca gregaria*). *Nature, New Biology* **233**, 53–55.

Durbin, R. P. (1961). Osmotic flow of water across permeable cellulose membranes. *J. gen. Physiol.* **44**, 315–326.

Gilbert, L. I. (1967). Lipid metabolism and function in insects. *Adv. Insect Physiol.* **4**, 69-211.

Goodhue, D. (1963). Some differences in the passage of food through the intestines of the desert and migratory locusts. *Nature, Lond.* **200**, 288–289.

Knowles, G. (1975). The reduced glucose permeability of the isolated Malpighian tubules of the blowfly *Calliphora vomitoria*. *J. exp. Biol.* **62**, 327–340.

Maddrell, S. H. P. & Gardiner, B. O. C. (1974). The passive permeability of insect Malpighian tubules to organic solutes. *J. exp. Biol.* **60**, 641–652.

Maddrell, S. H. P., Gardiner, B. O. C., Pilcher, D. E. M. & Reynolds, S. E. (1974). Active transport by insect Malpighian tubules of acidic dyes and acylamides. *J. exp. Biol.* **61**, 357–377.

Phillips, J. E. (1964). Rectal absorption in the desert locust, *Schistocerca gregaria* Forskal. I. Water. *J. exp. Biol.* **41**, 15–38.

Phillips, J. E. (1977). Excretion in insects: function of gut and rectum in concentrating and diluting the urine. *Fedn. Am. Socs. exp. Biol.* **35**, 2480–2486.

Phillips, J. E. & Dockrill, A. A. (1968). Molecular sieving of hydrophilic molecules by the rectal intima of the desert locust (*Schistocerca gregaria*). *J. exp. Biol.* **48**, 521–532.

Smith, J. N. (1962). Detoxication mechanisms. *A. Rev. Ent.* **7**, 465–480.

Treherne, J. E. (1957). Glucose absorption in the cockroach. *J. exp. Biol.* **34**, 478–485.

Treherne, J. E. (1958). The absorption of glucose from the alimentary canal of the locust, *Schistocerca gregaria* (Forsk.). *J. exp. Biol.* **35**, 297–306.

Treherne, J. E. (1967), Gut absorption. *A. Rev. Ent.* **12**, 43–58.

Weis-Fogh, T. (1952). Fat combustion and metabolic rate of flying locusts (*Schistocerca gregaria* Forskal). *Phil. Trans. R. Soc.* Ser. B. **247**, 1–36.