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THE REDUCED GLUCOSE PERMEABILITY OF THE ISOLATED MALPIGHIAN TUBULES OF THE BLOWFLY *CALLIPHORA VOMITORIA*

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SUMMARY

The excretion of several sugars by the isolated Malpighian tubules of *Calliphora vomitoria* has been investigated. The isolated tubules do not excrete glucose or trehalose at rates similar to those of sugars of the same molecular weight. Trehalose can be hydrolysed to glucose as it traverses the tubule wall. It is proposed that glucose can be reabsorbed by the tubule. Evidence is presented to suggest that glucose reabsorption can be saturated. Phloridzin was found to increase the rate of glucose excretion by the isolated Malpighian tubule.

INTRODUCTION

The permeability of Malpighian tubules of several insects to a range of organic solutes has now been investigated. Ramsay (1958) was the first to demonstrate conclusively that the isolated Malpighian tubules of *Carausius* were permeable to sugars and amino acids. In later papers, both Farquharson (1974) using the diplopod *Glomeris* and Maddrell & Gardiner (1974) using the insects *Rhodnius*, *Calliphora*, *Schistocerca*, *Manduca* and *Triatoma*, further demonstrated the permeability of the isolated Malpighian tubules of these species to organic solutes. Ramsay (1958) and Maddrell & Gardiner (1974), using *Carausius* and *Rhodnius* respectively, have provided evidence to suggest that sugars enter the tubule lumen by diffusion. *Glomeris* may differ from insects in that their Malpighian tubules permit the passage of polydextrans of molecular weight $\leqslant$ 18000 (Farquharson, 1974). It is difficult to conceive of polydextran molecules diffusing across the tubule wall of *Glomeris*.

Ramsay (1958), Maddrell (1971), and Maddrell & Gardiner (1974) recognized that if the tubules were permeable to sugars and amino acids, there could be a potential loss of useful metabolites from the haemolymph. Maddrell (1971) and Maddrell & Gardiner (1974) also stressed that the permeabilities of the Malpighian tubules and hindgut epithelia ought to be matched if the overall control of organic solute loss is to be effective. These same authors considered that recovery of desirable organic solutes could occur across either the hindgut (sometimes called ileum) or rectum. Indeed, Balshin & Phillips (1971) demonstrated the recovery of glycine by the rectum of *Schistocerca*. Wall & Oschman (1970) came to the same conclusions when working with the

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cockroach. However, Phillips & Dockrill (1968) showed that the cuticular lining from the rectum of *Schistocerca* was impermeable to certain organic solutes which were likely to occur in the tubule fluid. Maddrell & Gardiner (1974) generally favour the anterior hindgut or ileum as an appropriate site for organic solute recovery.

In spite of these recent developments, there has been no evidence to show that the important haemolymph sugars, such as glucose and trehalose, are present in the tubule fluid at levels comparable to those of non-metabolizable sugars. This investigation provides evidence to suggest that some sugars have only limited entry to the tubule lumen.

MATERIALS AND METHODS

Adult flies were reared from maggots which were purchased from suppliers. Malpighian tubules from male flies were isolated into medium droplets. The procedures were similar to those of Taylor (1970). The compositions of the bathing media used, were based upon those of Berridge (1966). Unless otherwise stated, individual sugars were usually added to the Ringer solutions at a carrier concentration of 50 mM. The following sugars and related substances were used:- glycerol, erythritol, xylose, sorbose, L-glucose, D-glucose, maltose, sucrose, trehalose and methyl inulin (bathing medium concentration of inulin was not 50 mM but was present as a saturated solution). Not all the above sugars would support fluid secretion; 10 mM D-glucose was therefore added, in each case, to ensure the presence of a sugar which could support fluid formation. To these solutions, 50 μCi of ^{14}C isotope (universally labelled) were dissolved in 1·5 ml of Ringer. Isotopes were supplied by the Radiochemical Centre, Amersham. Bacterial and fungicidal breakdown of sugars was reduced by adding genticin and amphotericin. Laskey (1970) used these agents to decontaminate amphibian egg cultures.

The isolated tubules were allowed to secrete fluid for set time periods. Bathing medium and tubule fluid samples were then taken into finely-pulled capillaries and transferred to a paraffin oil dish where the diameter of each sample droplet was measured using a moving hairline eye-piece. From these diameters the droplet volumes were calculated. The radioactivities of the tubule fluid (TF) and bathing medium (M) samples were counted on an I.D.L. end-window, anti-coincidence, Geiger Müller counter.

In some cases, samples were examined by thin-layer chromatography (T.L.C.). The plates were supplied by Carl Schleicher and Schüll. The solvent system, butanol: acetic acid: water (40:15:15 v/v) was found most convenient for the separation of glucose and trehalose solutions. The T.L.C. plates were developed for 3 h in the solvent system, strips were then scanned by an I.D.L. 2029 chromatogram scanner. The positions of the radioactivity peaks for labelled glucose and trehalose were $1\frac{1}{2}$ and 1 in. respectively from a standard point on the T.L.C. strip.

To investigate the movement of sugars from the tubule lumen to the haemolymph, the double droplet arrangement of Ramsay (1958) was chosen (Fig. 1). An isotopically labelled medium droplet containing either glucose or sorbose was used for the first medium droplet (M_1). The second medium droplet (M_2) consisted of a Ringer solution containing either unlabelled glucose or sorbose at the same concentrations as that thought to be present in the tubule lumen. After the tubule had secreted for

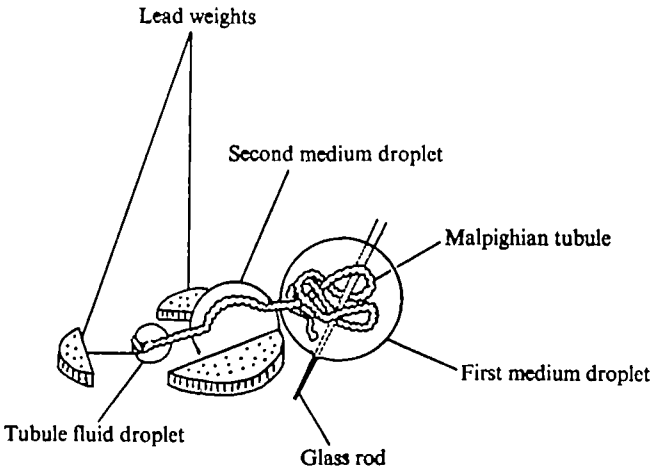

Fig. 1. The double droplet arrangement.

2½ h, the second medium droplet and tubule fluid samples were taken and their radioactive content counted. The media droplets were replaced with the other Ringer solutions; e.g. if glucose was used for the first 2½ h, sorbose was used for the second period.

Results for these experiments are expressed as a reabsorption ratio, which is defined as the fraction of isotope passing into the second medium droplet over the total amount of isotope passing from the first medium droplet in the tubule fluid. Thus, if:

b = total counts in second medium droplet, M_2,
d = total counts in tubule fluid sample, TF,

then the reabsorption ratio R equals:

$$R = \frac{b}{b+d}.$$

During the double droplet experiments two batches of isotope were used, the second batch being made up to a higher specific activity than the first.

RESULTS

The ability of isolated tubules to utilize different sugars as an energy source to promote fluid secretion has already been tested by Berridge (1966). However, L-glucose has not been used previously and preliminary experiments were performed to see if the tubule would utilize this sugar (Fig. 2). After replacement of D-glucose by L-glucose at 90 min, there is a period of 30 min in which fluid secretion continues and then the rate of fluid secretion falls until D-glucose is readministered at 150 min. Presumably, during the period of exposure to L-glucose when fluid secretion continues, the tubule is utilizing endogenous energy reserves.

(*a*) *The* TF/M *radioactivity ratios*

The *TF/M* ratios for a range of sugars are shown in Table 1 and Fig. 3. The sugars are listed in order of increasing molecular weight. The results show that as the molecular weight increases, the *TF/M* ratio decreases until an apparent steady level is reached.

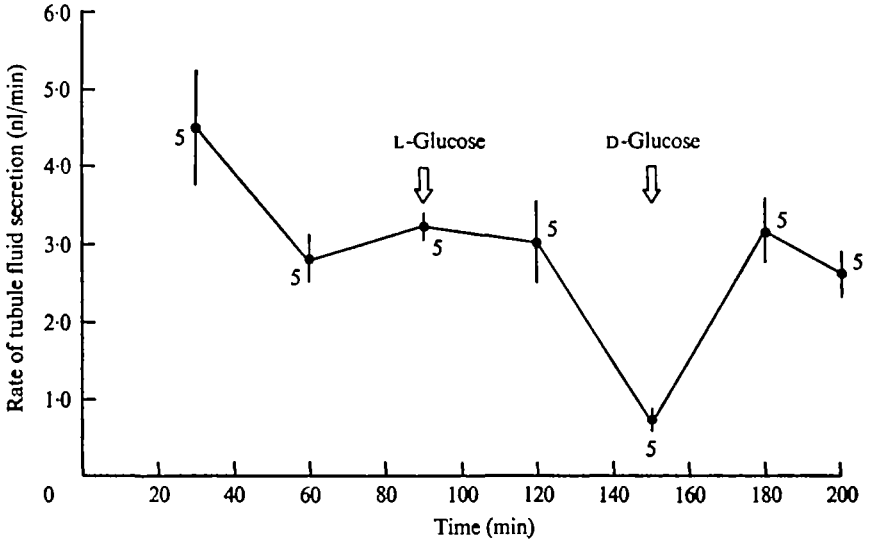

Fig. 2. The effect of replacing D-glucose with L-glucose (shown by arrow heads) upon the rate of fluid production by the isolated Malpighian tubule. (In all figures the means are given ± one standard error, numbers adjacent to means refer to the total number of observations from which that mean was calculated.)

Table 1. *The radioactivity ratio* (TF/M) *for several sugars and inulin in relation to their molecular weight and hydrated molecular diameter*

Sugar	Molecular weight	Hydrated molecular diameter (Å)	*TF/M*	S.E.	Rate of fluid production nl/min	S.E.	*n*
Glycerol	92	2·75	0·96	0·058	2·22	0·42	10
Erythritol	124	–	0·94	0·054	1·38	0·15	8
Xylose	150	3·60	0·74	0·024	1·60	0·25	9
Sorbose	180	4·25	0·63	0·040	1·25	0·17	11
L-Glucose	180	4·25	0·58	0·045	2·27	0·16	12
D-Glucose	180	4·25	0·25	0·040	2·02	0·37	10
Maltose	360	5·25	0·27	0·044	1·84	0·33	10
Sucrose	342	5·25	0·25	0·022	1·06	0·17	7
Trehalose	342	5·25	0·079	0·017	1·44	0·29	7
Inulin	5200	—	0·25	0·050	1·00	0·13	5

This level is represented by the value found for inulin which is about 0·25 and is similar to the sucrose and maltose values.

The 'metabolically inert' monosaccharides sorbose and L-glucose have ratios of about 0·6, which are significantly different ($P < 0{\cdot}01$) from the D-glucose ratio of 0·25. It is clear that, in some manner, the passage of D-glucose across the tubule wall is restricted; possibly the Malpighian tubule is reabsorbing glucose by a mechanism similar to that found in vertebrate and crustacean nephrons. Similarly, the permeability of trehalose is reduced in comparison to other disaccharides, whether they be metabolizable (e.g. maltose) or 'metabolically inert' (e.g. sucrose).

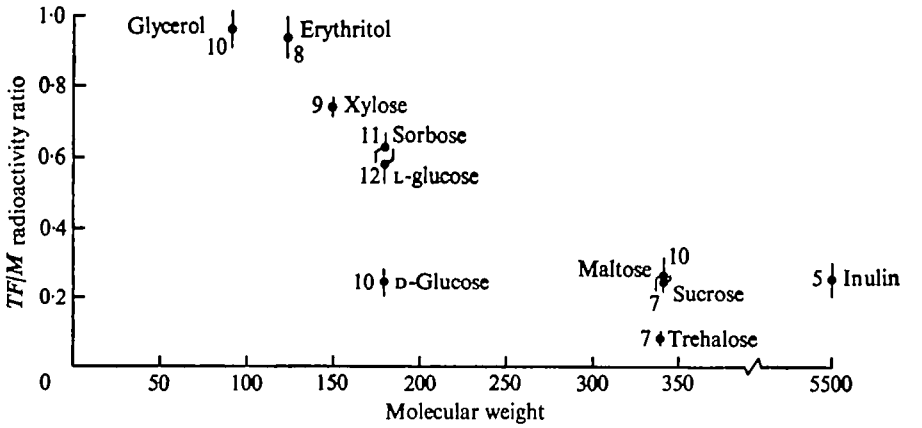

Fig. 3. The relationship between the *TF/M* radioactivity ratio and the molecular weight of various sugars.

(b) Chromatography of fluid samples

It is important to establish the chemical identity of the radioactive molecules which are present in the fluid samples. The radioactive peaks of the bathing medium in which tubules were incubated for 3 h in [^{14}C]glucose correspond to the radioactivity peaks for normal, control Ringer. The peaks for the tubule fluid samples are in similar positions to the bathing medium samples (Fig. 4). There is, possibly, for the tubule fluid samples, another small amount of radioactivity present at a position about 3 in. from the spot origin. However, this amount of radioactivity is small compared with that present in the glucose peak. These results indicate that there is negligible metabolism of D-glucose as it traverses the tubule wall. The *TF/M* ratio, based upon the chemical concentrations represented in Fig. 4, can now be calculated (Table 2). The mean glucose *TF/M* ratio for the chromatographed samples was $0{\cdot}27 \pm 0{\cdot}016$ (S.E.); this is not significantly different ($P > 0{\cdot}1$) from the non-chromatographed glucose *TF/M* ratio of $0{\cdot}25 \pm 0{\cdot}04$ (S.E.). Therefore, the glucose ratio of the non-chromatographed samples represents the true glucose chemical concentration ratio. It must be emphasized that the case is only proven when the glucose concentration in the bathing medium is 50 mM.

When tubules are incubated in [^{14}C]trehalose the radioactivity in the bathing medium is retained in the trehalose molecule (Fig. 5). However, in contrast to the glucose experiments, the tubule fluid radioactivity appears to be split between the trehalose and glucose zones (Fig. 5). This implies that as trehalose traverses the tubule wall a certain amount of disaccharide is hydrolysed to glucose.

The results shown in Fig. 5 are too variable and too scanty to permit estimation of the rates of trehalose hydrolysis. The differing abilities of Malpighian tubules to split trehalose and possibly reabsorb glucose could also contribute to the variability of the results. For instance, tubule fluid sample 2 of Fig. 5 appears to have a high glucose content; this could be due to either slow glucose reabsorption or to a very permeable tubule coupled with rapid trehalose hydrolysis.

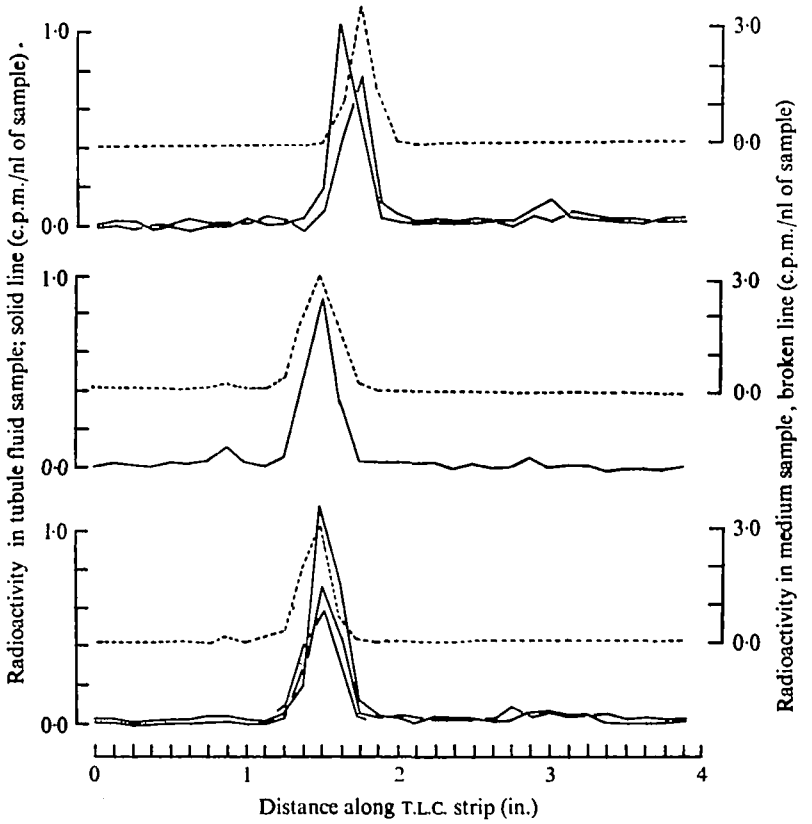

Fig. 4. The radioactivity levels (c.p.m./nl. of sample) of the tubule fluid (solid line), and bathing medium (broken line) along the T.L.C. strip after the tubules had been incubated in 50 mM [^{14}C]glucose.

(c) Double droplet experiments

It has been proposed that glucose may be recovered from the tubule lumen. The only evidence for this is the low *TF/M* ratio of glucose compared with other monosaccharides. To obtain more direct evidence, the double droplet arrangement of Ramsay (1958) was adopted. The procedure has been described in the Materials and Methods. Essentially, the experiment compared the movement of ^{14}C radioactivity of sorbose and glucose from the tubule lumen to the bathing medium. Experimental conditions were chosen to minimize chemical concentration gradients from the lumen to second medium droplets.

The mean glucose reabsorption ratio is significantly higher (P between 0·01 and 0·02) than the corresponding sorbose value (Table 3). This is to be expected if the tubule was preferentially moving glucose from the tubule lumen to haemolymph. The normal *TF/M* radioactivity ratios for sorbose and glucose are generally lower than when the tubules are placed in single medium droplets. This is perhaps because the tubule fluid samples contained some fluid secreted from the very low specific radioactivity of the second medium droplet. Consequently, the volume of tubule fluid-sampled should be reduced by that volume of fluid secreted from the second medium droplet. This correction has not been made since the volumes of fluid

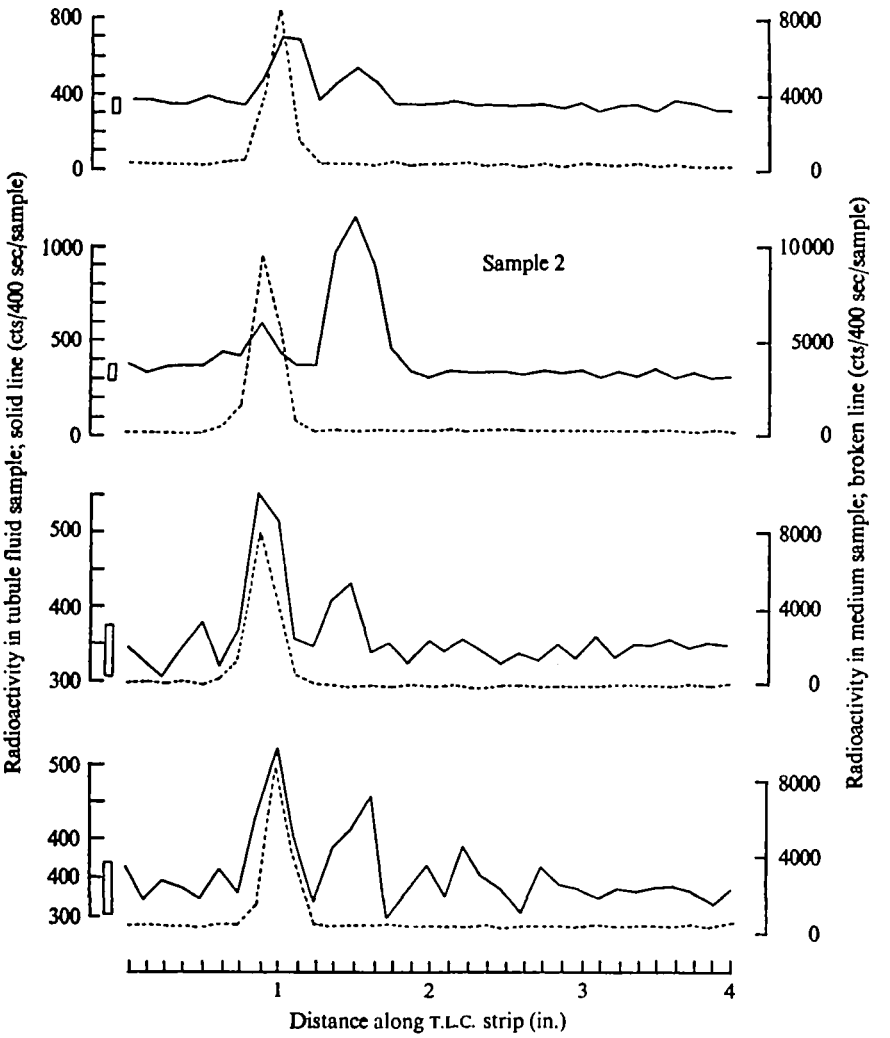

Fig. 5. The radioactivity levels (cts/400 sec/sample) of tubule fluid (solid line) and bathing medium (dotted line) samples along the T.L.C. strip after the tubules had been incubated in 50 mM [^{14}C]trehalose. The mean background rate was 335 cts/400 sec; the standard deviation (S.D). of this count would be $\sqrt{335}$ or 18·3 cts and the mean $\pm 1{\cdot}96 \times$ S.D. would encompass 95 % of all counts. The 95 % confidence limits are denoted by the vertical bars on the left side of this figure.

secreted from M_2 were not measured. However, the mean radioactivity ratios for sorbose and glucose from the double-droplet experiments are significantly different ($P < 0{\cdot}01$). The mean specific radioactivities of the droplets were:

M_1 4083 ± 106 (S.E.) c.p.m./μl,
M_2 12 ± 4 (S.E.) c.p.m./μl,
TF 582 ± 27 (S.E.) c.p.m./μl.

(d) The effects of the bathing medium glucose concentration on the TF/M *ratio*

If the Malpighian tubule is reabsorbing glucose it may be possible to increase the glucose ratio to a value similar to that of sorbose or L-glucose. Therefore, the effect

Table 2. *The radioactivity in the glucose zones of the tubule fluid and bathing medium samples from Fig. 4. Bathing medium contained* ^{14}C*-(U)-glucose*

c.p.m./nl		
Tubule fluid	Bathing medium	*TF/M* ratio
1·67	6·11	0·27
1·39		0·23
1·69	6·76	0·25
2·06	5·88	0·35
1·43		0·25
1·36		0·26
		0·27 ± 0·016 (S.E.)

Table 3. *The activities in droplets* M_1, M_2 *and the secreted tubule fluid droplet TF from the double droplet experiments. The reabsorption ratios are also given (means are expressed as* ± 1 × S.E.)

	Activity in droplet M_1 (c.p.m./nl) (*a*)	Activity in droplet M_2 (total cts/100 sec) (*b*)	TF volume (*c*)	Activity in tubule fluid (TF) droplet (cts/100 sec) (*d*)	Specific activity of tubule fluid (c.p.m./nl) $0{\cdot}6\left(\frac{b+d}{c}\right)=e$	Reabsorption ratio $\frac{b}{b+d}$	Activity ratio $\frac{e}{a}$
Sorbose	2·36	580	1141	1782	1·24	0·25	0·52
	2·97	1405	1292	1576	1·38	0·47	0·46
	1·72	118	676	596	0·64	0·16	0·37
	1·75	291	418	288	0·83	0·50	0·47
	3·43	599	463	756	1·76	0·44	0·51
	4·37	822	504	330	1·37	0·71	0·31
	4·25	354	1238	1543	0·92	0·19	0·22
						0·39 ± 0·069	0·41 ± 0·039
Glucose	2·95	215	479	215	0·54	0·50	0·18
	3·08	519	662	289	0·73	0·64	0·24
	2·37	166	788	289	0·35	0·36	0·15
	3·69	514	691	85	0·52	0·86	0·14
	3·96	736	374	202	1·50	0·78	0·38
	4·04	516	693	163	0·59	0·76	0·15
	4·26	377	618	220	0·58	0·63	0·14
	4·12	590	501	218	0·97	0·73	0·23
						0·66 ± 0·054	0·20 ± 0·027

of increased medium glucose concentration on the level of glucose in the tubule fluid was investigated.

A series of isolated tubule preparations was set up with a bathing medium glucose concentration varying from 5 to 200 mM. It is clear that as the medium glucose concentration increases, the *TF/M* ratio also increases (Fig. 6). For comparison, there is a significant decrease ($P < 0{\cdot}05$) in the radioactivity ratio of sorbose as the sorbose bathing medium concentration is increased from 15 to 50 mM. The differences between the sorbose and glucose ratios suggests that *TF/M* ratios are affected by the bathing medium sugar concentration. The maximal glucose ratio obtained in these experiments does not reach the sorbose ratio when measured at a bathing medium

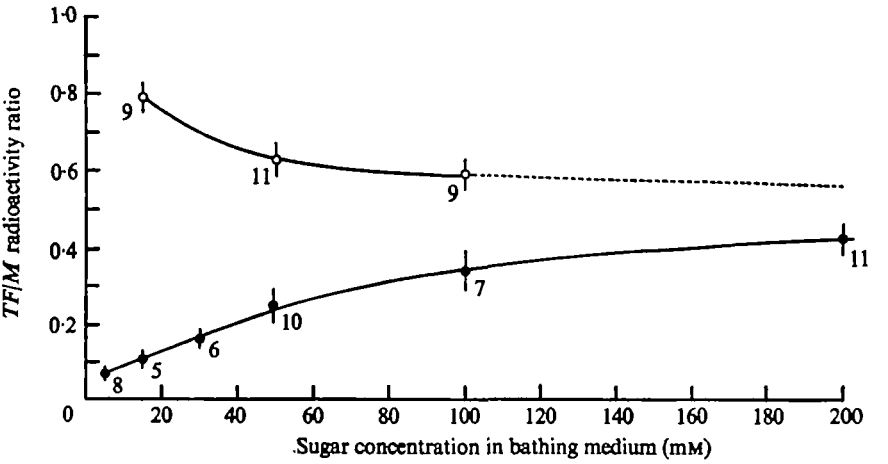

Fig. 6. The effect of the bathing medium glucose (●—●) or sorbose (○—○) concentration upon the *TF/M* ratio.

concentration of 100 mM, although extrapolation of the sorbose results to medium concentrations in excess of 100 mM shows that the monosaccharide *TF/M* ratios may converge.

Ramsay (1958) and Maddrell & Gardiner (1974) suggested that one criterion for the acceptance of substances diffusing across the Malpighian tubule wall was that the *TF/M* ratio should be independent of the bathing medium concentration. In the case of glucose, the evidence presented above shows that this criterion is not met.

At normal haemolymph glucose concentrations of about 10 mM the tubule fluid glucose concentration would be very small (approximately 1 mM). Hydrolysis of haemolymph trehalose may elevate the tubule fluid glucose concentration.

(*e*) *The effect of phloridzin upon the glucose* TF/M *ratio*

It is now apparent that the isolated tubule possesses some mechanism to restrict the loss of glucose and trehalose. It is well known that phloridzin or one of its metabolites inhibits glucose transport in vertebrate tissues (Diedrich, 1966, 1968). Phloridzin has also been shown to affect the renal excretion of glucose in both crustaceans (Binns, 1969) and molluscs (Potts, 1967).

Preliminary experiments revealed that phloridzin, at a concentration of 1 mM, caused an immediate increase in the rate of fluid secretion of isolated Malpighian tubules – a curious and unexpected effect (Fig. 7). Another set of experiments was performed with [^{14}C]D-glucose (50 mM) and phloridzin (1 mM) in the bathing medium. Thus, in these experiments, the tubule was subjected to phloridzin from the beginning of the experiment rather than substitution at 90 min. The effect of phloridzin on the glucose ratio is shown in Fig. 8, Table 4. Fig. 8 includes the control experiments, where the gradual rise in the sorbose and glucose ratios are plotted when phloridzin was omitted from the bathing media. The results show that phloridzin does affect the glucose ratio and that the ratio, as predicted, approaches that of sorbose. This is made clear by comparing the glucose ratios at 240 min (Table 5). It can be seen

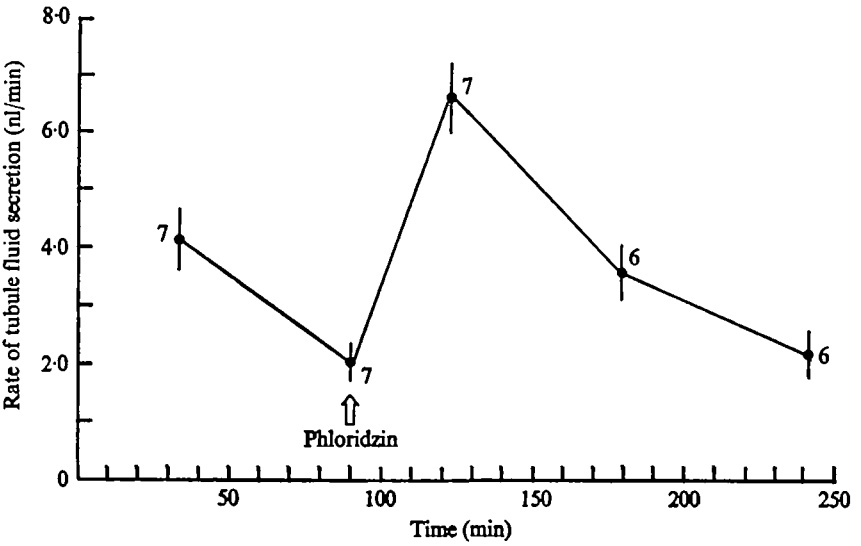

Fig. 7. The effect of 1 mM phloridzin upon the rate of fluid secretion by the isolated Malpighian tubule.

Table 4. *The effect of phloridzin* (1 mM) *upon the glucose TF/M radioactivity ratio. Bathing medium glucose concentration was* 50 mM

Time (min)	*TF/M*	S.E.	Rate of tubule fluid secretion (nl/min)	S.E.	*n*
30	0·11	0·03	7·0	0·81	10
60	0·19	0·05	3·6	0·29	10
120	0·32	0·06	2·8	0·58	10
180	0·47	0·07	2·1	0·27	10
240	0·59	0·07	1·4	0·21	6

that, although the rates of fluid secretion are similar ($P > 0{\cdot}1$), the glucose ratios are significantly different ($P < 0{\cdot}01$).

Ramsay (1958) and Maddrell & Gardiner (1974) have shown that if an organic solute crosses the tubule wall by diffusion alone, then the *TF/M* ratio should be related to the rate of tubule fluid secretion in the following manner:

$$TF/M = \frac{k}{r+k}, \tag{1}$$

or

$$M/TF = \frac{r}{k}+1,$$

where r = rate of tubule fluid secretion per mm^2 of tubule surface area, which for *Calliphora* is 4 mm^2 (value given by Maddrell & Gardiner, 1974), and k = a permeability constant.

The above relationship is only valid for substances diffusing across the tubule wall. For instance, the bulk movement of molecules in solution, such as may occur through water-filled pores, has been ignored.

If phloridzin has no effect upon the tubule except to increase the rate of fluid

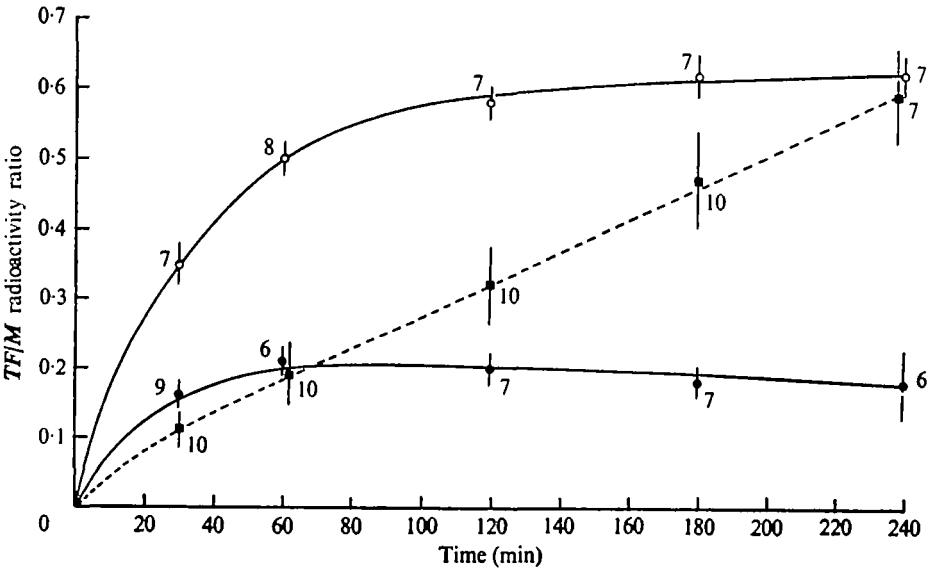

Fig. 8. The effect of 1 mM phloridzin upon the glucose ratio (■ - - - - ■). Glucose concentration in the bathing medium was 50 mM. Glucose ratio without phloridzin (●—●); sorbose ratio without phloridzin (○—○).

Table 5. *A comparison of* D-*glucose* TF/M *radioactivity ratios after* 240 *min, with and without phloridzin*

	TF/M	S.E.	Rate of tubule fluid secretion (nl/min)	S.E.	*n*
Glucose with phloridzin omitted	0·18	0·05	1·87	0·42	6
Glucose with phloridzin present	0·59	0·07	1·40	0·22	6

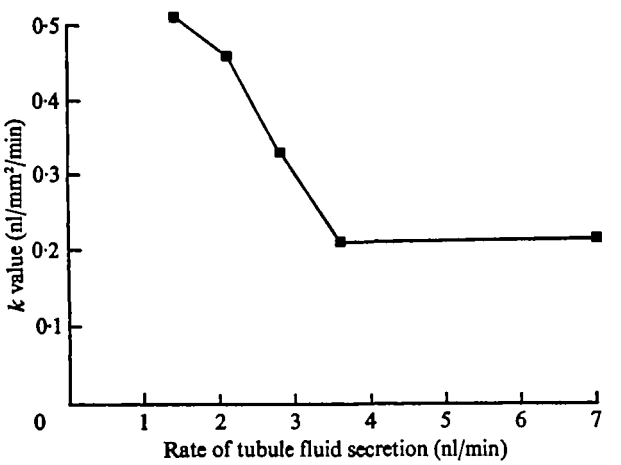

Fig. 9. The glucose permeability constant, *k*, in relation to the rate of tubule fluid secretion which has been elevated due to the presence of 1 mM phloridzin.

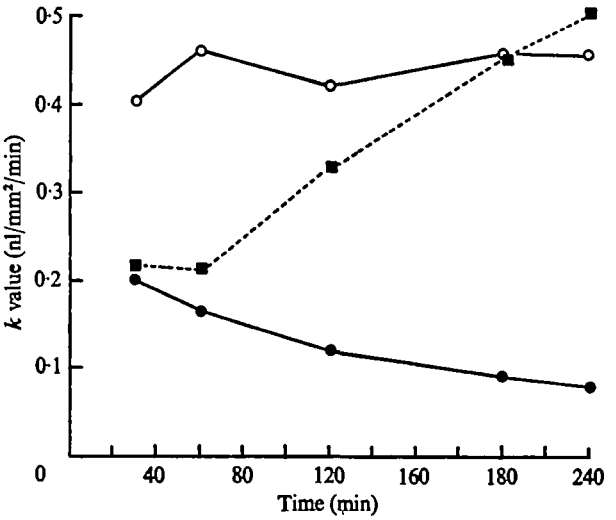

Fig. 10. The effect of 1 mM phloridzin upon the glucose permeability constant (■---■) when plotted against time. The permeability constants of sorbose (○—○), and glucose (●—●) without phloridzin when plotted against time.

secretion, then the glucose ratio should vary according to equation (1) and the constant k should not vary at all. The values for TF/M and rates of fluid secretion given in Table 4 have been substituted into equation (1) and the k values calculated. These are plotted in relation to the rates of tubule fluid secretion (Fig. 9). Since, after the addition of phloridzin, the rate of fluid secretion is related to time, then the values of k in Fig. 9 can be related to the time after phloridzin application (Fig. 10). Values of k, calculated from the TF/M ratios (Fig. 8) and rates of fluid secretion in the presence of sorbose and glucose alone, are plotted as control values in Fig. 10. If the varying TF/M glucose ratios of Fig. 8 were only due to the effects of the falling rates of fluid secretion, then one would not expect the glucose k values to increase. This treatment of the results indicates that phloridzin increases the glucose permeability of the tubule. The constant k represents the overall glucose permeability of the tubule, which, if glucose reabsorption is occurring, can be considered to comprise two components, i.e.

$$k = \phi - \text{reabsorption},$$

where ϕ = a second permeability constant.

Consequently if phloridzin increased the value of k, then either the value of ϕ has been increased or reabsorption reduced. If phloridzin is acting in a similar manner as in other invertebrate excretory systems (Potts, 1967; Binns, 1969), then it is presumed to be reducing glucose reabsorption rather than increasing the value of ϕ.

DISCUSSION

The evidence, taken as a whole, demonstrates that the isolated Malpighian tubules of the blowfly markedly restrict the output of the two major haemolymph sugars. It is important to assess how such mechanisms may operate in the intact fly. In insects, the haemolymph glucose concentration is usually regulated at a very low level, around 10 mM, and the trehalose concentration around 60 mM (Clegg & Evans, 1961). Conse-

quently, the greatest potential energy losses from sugars could arise from trehalose diffusing into the tubule fluid. Thus, any system which is designed to mitigate sugars losses from the fly, must account for the disadvantages of both glucose and trehalose excretion.

Since the haemolymph glucose levels are low, then as the internal tubule cell level of glucose rises, due to trehalose hydrolysis and glucose reabsorption, diffusion gradients will develop from cell to haemolymph. In this way the larger haemolymph volume could act as a 'glucose sponge' and stabilize the Malpighian tubule cell glucose concentration. The recovery of glucose by this method may be called 'diffusion recovery'. In this respect, the lower the rate of fluid secretion, the easier it becomes for the tubule to establish favourable diffusion gradients.

It is interesting to note that Dahlman (1970) has estimated the trehalase content of larval tissue of the tobacco hornworm *Manduca secta*. He found that the Malpighian tubules had the highest titre of trehalase. The distal portion of the tubules had the greatest activity and the author suggests that, since the Malpighian tubules have a cryptonephridial arrangement, the high trehalase activity is required to fund the energy-dependent ionic fluxes which occur in these tissues.

The precise cellular localization of trehalase in insect cells has been investigated but the conclusions are sometimes contradictory, a situation recognized by Sacktor (1970). However, Douglas & Sacktor (1971) localized the particulate trehalase of *Calliphora* thoracic muscles in the mitochondrion, specifically the inner mitochondrial membranes. If the mitochondria of the flies' Malpighian tubules also contain trehalase, then the cell boundaries of the tubule which abound in mitochondria (Berridge & Oschman, 1969) would be ideal for trehalose metabolism. Berger & Sacktor (1970) have also localized mammalian trehalase in the brush-border of rabbit kidney.

'Diffusion recovery' would not lead to a lowered TF/M glucose ratio, because the glucose diffusion gradients are always from the bathing medium to the tubule lumen. 'Diffusion recovery' is possible only when the lumen glucose concentration is above that of the haemolymph, but glucose reabsorption may operate at lumen concentrations below the haemolymph. The relative contributions of both types of glucose recovery cannot at present be fully assessed. It would be presumptive to label glucose reabsorption as active reabsorption, since there is only limited evidence to correlate it with other, more accepted examples of active glucose transport. These have been discussed by Smyth (1971). The experiments which demonstrated the saturable nature of glucose reabsorption and its inhibition by phloridzin go some way to characterize the peculiar glucose metabolism of isolated Malpighian tubules.

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