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GLUCOSE TRANSPORT BY SHORT LOOPS OF HENLE IN THE RAT

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SUMMARY

1. Short loops of Henle were artificially perfused with saline solutions containing 5 or 0 mM-glucose in the presence and absence of phlorizin.

2. Net fluid reabsorption was greater when glucose but no phlorizin was present than in all other series.

3. Glucose was reabsorbed from glucose-containing perfusate and this was abolished by phlorizin. Secretion of glucose occurred into the perfusate which initially contained no glucose and this secretion was enhanced by phlorizin.

4. Sodium reabsorption was inhibited by phlorizin when glucose was present, but enhanced by phlorizin when glucose was absent.

5. It can be shown that there is secretion of some osmotically active solute in all series. Its secretion is enhanced by phlorizin in the absence of glucose.

INTRODUCTION

Under physiological circumstances, most of the glucose filtered at the glomerulus of the kidney is reabsorbed in the first part of the proximal tubule (Fröhnert, Höhmann, Zwiebel & Baumann, 1970; Bishop, Green & Thomas, 1979). As a consequence, data on the transport of glucose by other parts of the nephron are sparse and the interaction between glucose and other transported species has been considered only in relation to the proximal convoluted tubule.

That there is normally some glucose in the urine is now generally accepted (see references in Bishop, Elegbe, Green & Thomas, 1978*a*) although this is at low concentration and only about 0·5% of the filtered load. In a micropuncture study of glucose reabsorption in the rat, fluid from the end of the proximal convoluted tubule was found to contain 3–5% of the filtered load (Bishop *et al.* 1979). Taken together, these observations suggest that some glucose reabsorption occurs beyond the end of the proximal convoluted tubule even under normal physiological circumstances. A glucose-reabsorptive capacity of more distal segments is also suggested by studies in animals infused with a low dose of phlorizin, where urine contained 30% of the filtered load (Bishop *et al.* 1978*a*) but the fluid at the end of the proximal convoluted tubule contained 99% of the filtered glucose (Bishop *et al.* 1979).

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The sites of this further reabsorption are not known with certainty. Tune & Burg (1971) demonstrated that the pars recta of rabbit proximal tubules could reabsorb glucose, but that the capacity of this segment was only 20% of that of the proximal convoluted tubule; also, in a study on microperfused short loops of Henle, von Baeyer (1975) calculated transport constants for glucose reabsorption, assuming that all the glucose was reabsorbed by the pars recta of the proximal tubule. On the other hand, there have been reports that both the loop of Henle (Bishop & Green, 1979*b*) and more distal parts of the nephron (Bishop & Green, 1979*a*) also have the capacity to reabsorb glucose.

In recent investigations (Bishop *et al.* 1978*a*; Bishop, Green & Thomas, 1978*b*; Bishop *et al.* 1979) on the glucose transport characteristics of the proximal convoluted tubule in the rat, we reported that there was no major quantitative effect of glucose on sodium and water transport, and that phlorizin, generally considered to be a specific inhibitor of glucose transport (Frasch, Fröhnert, Bode, Baumann & Kinne, 1970), had effects on water and ion fluxes independent of those on glucose. But again, there is a lack of corresponding information about more distal nephron segments.

Accordingly, this study was designed to provide information on glucose transport and the effects of glucose and phlorizin on water and sodium transport by short loops of Henle, which are accessible to micropuncture, perfused with artificial solutions with an ionic composition resembling end proximal tubular fluid. The concentration of glucose chosen (5 mM) was such that there was little likelihood of its passive reabsorption.

METHODS

Male Sprague-Dawley rats of 140–180 g were prepared for micropuncture as previously described (Bishop *et al.* 1978*b*). 0·9% saline was infused into the jugular vein at 20 μl. min^{-1} after a priming dose of 1 ml. to compensate for fluid losses during surgery.

Short loops of Henle were perfused in the following way. Randomly selected proximal tubules were punctured with a sharpened micropipette (external diameter 10–12 μm) containing a 1% solution of the dye Lissamine Green and a small bolus injected. This allowed identification of the last surface proximal loop and, after a further 15–30 sec, the first distal convolution on the kidney surface. When both of these sites were considered to be accessible to micropuncture, a second pipette, connected to a perfusion system (see Bishop *et al.* 1978*b*), was inserted into the last proximal loop and perfusion commenced at 25 nl. min^{-1}. The first pipette was withdrawn and replaced by one containing castor oil stained with Sudan Black, which was injected to fill the convolutions between the two pipettes. This third pipette was then removed, allowing egress of proximal tubular fluid, and used to puncture the previously identified surface distal convolution; a long distal oil block was inserted. Fluid was perfused from the second pipette through the loop of Henle and collected into this re-sited third pipette.

Four series of experiments were performed with different perfusion fluids (no. of animals is given in Table 1 and no. of perfused tubules in Table 2):

Series A: the perfusion fluid contained (mM) NaCl, 151; KCl, 4; $CaCl_2$, 2; Na_2HPO_4, 2; KH_2PO_4, 0·5; glucose 5. [^{3}H]inulin 50 μc ml.$^{-1}$ and Lissamine Green (to a final concentration of 0·05%) were added and the whole gassed with 95% O_2/5% CO_2.

Series B: the perfusion fluid was the same as Series A with the addition of phlorizin (0·1 mM; K & K Rare Chemicals, Liverpool).

Series C: the perfusion fluid was similar to Series A except that the glucose was replaced by an osmotically equivalent amount of NaCl.

Series D: the perfusion fluid was the same as Series C with the addition of phlorizin (0·1 mM).

The volume of the collected perfusate was measured in a calibrated capillary and a measured aliquot taken for estimation of [^{3}H]inulin concentration in a liquid scintillation counter with PCS

(Radiochemical Centre, Amersham) as the scintillant. The inulin concentration in the collected fluid was compared with that of a similar aliquot of perfusate. The rate of perfusion was checked after each experiment and only when the quantity of inulin recovered was 90–110% of that perfused was the tubule included in the study. Sodium and potassium concentrations in the collected fluid and the perfusate were measured on a Helium Glow photometer (Aminco Inc., Silver Springs, Maryland) and osmolality on a nanolitre osmometer (Clifton Technical Physics, Hartford, N.Y.). Glucose was determined fluorometrically using an enzymatic technique previously described (Bishop *et al.* 1978*b*). Attempts were made to fill the tubules with silicone rubber but these were, in the main, unsuccessful.

At the end of the experiments, samples of blood were collected from a tail vein, the plasma was separated and analysed for sodium and potassium (EEL 150 Clinical Photometer) and osmolality (Advanced Osmometer).

Calculations

$$\text{Net reabsorption of water, } V = V_0(1-(\text{In}_0/\text{In}_1)).$$
$$\text{Net flux of any other constituent, } \Phi_A = V_0\,(A_0-A_1(\text{In}_0/\text{In}_1)),$$

where V_0 is the perfusion rate, In_0 is the inulin concentration in the perfusate and In_1 that in collected fluid, A_0 the concentration of any substance in the perfusate and A_1 its concentration in the collected fluid.

The 'osmotic deficit' (see Results and Discussion) was calculated as:

$$\text{Osmolality}-(1{\cdot}86[\text{Na}+\text{K}]+[\text{glucose}]).$$

Results presented are means ± standard error of the mean; and Student's t test, paired or unpaired, was used to assess the significance of any difference between perfusate and collected fluid and between series, respectively.

RESULTS

All rats maintained normal blood pressure throughout the experiment and in no case was the mean blood pressure less than 100 mmHg. There were no significant differences of blood pressure between the different series. The composition of plasma samples taken from a tail vein at the end of the experiment, together with the composition of the tubular perfusates measured in the same way as the tubular fluid samples, is given in Table 1; there were no significant differences between any of the series as regards sodium, potassium, glucose or osmolal concentrations.

The concentrations of solutes in the collected fluid are given in Table 2, together with the perfusion rate, the inulin concentration ratios and the percentage of inulin that was recollected. Inulin ratios (which reflect water reabsorption) were similar except for Series A (perfusate containing glucose) where the ratio was significantly greater than in all other series ($P < 0{\cdot}001$ in all cases). Correspondingly, the net water reabsorption was greater in this series as shown in Fig. 1; with glucose perfusate (Series A), mean reabsorption was 6·5 nl . min^{-1} whereas the mean reabsorption for the others (Series B–D) ranged from 4·5 to 4·8 nl . min^{-1} ($P < 0{\cdot}001$ for all cases).

Glucose was reabsorbed from the glucose-containing perfusate (Series A) and the concentration in collected fluid, 2·96 ± 0·21 mM, was significantly less than that perfused ($P < 0{\cdot}001$; cf. Tables 1 and 2). The net flux of glucose in this series (Fig. 2) indicates that the loop has a considerable ability to reabsorb glucose when exposed to this quantity of glucose at the unphysiological concentration of 5 (mM). Reabsorption was abolished by the addition of phlorizin (Series B) when the net flux of glucose was not significantly different from zero (Fig. 2). In Series B, the significant

($P < 0.005$) increase in glucose concentration (to 5.59 ± 0.10 mM) above that measured in the perfusate (4.9 ± 0.1 mM) was due to water abstraction. When glucose was absent from the perfusion fluid (Series C), net secretion into the perfusate occurred, as has previously been described for proximal tubule (Bishop *et al.* 1978*b*); the net secretory flux (8.1 ± 1.1 p-mole min^{-1}) was significantly greater than zero ($P < 0.001$; Fig. 2)

TABLE 1. Plasma composition and measured perfusate concentrations

	Series	A	B	C	D
		Glucose	Glucose + phlorizin	No glucose	No glucose + phlorizin
	n	6	7	8	6
Sodium (mM)	Plasma	147 ± 4	144 ± 4	145 ± 3	147 ± 3
	Perfusate	151 ± 2	150 ± 2	154 ± 2	155 ± 2
Potassium (mM)	Plasma	5.2 ± 0.3	4.9 ± 0.5	5.2 ± 0.5	5.4 ± 0.6
	Perfusate	4.3 ± 0.3	4.5 ± 0.2	4.8 ± 0.3	4.3 ± 0.1
Glucose (mM)	Plasma	4.7 ± 0.5	5.1 ± 0.5	4.9 ± 0.6	5.5 ± 0.4
	Perfusate	4.7 ± 0.2	4.9 ± 0.1	0	0
Osmolality (m-osmole kg^{-1} water)	Plasma	298 ± 2	306 ± 6	300 ± 5	303 ± 3
	Perfusate	298 ± 6	299 ± 7	298 ± 5	299 ± 2

n = no. of animals.

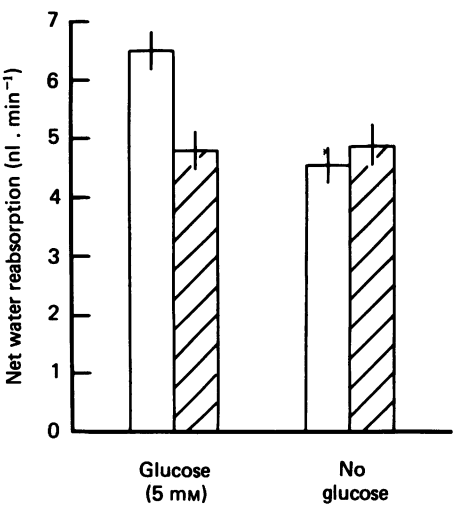

Fig. 1. Net water reabsorption in short loops of Henle perfused with fluid containing 5 or 0 mM-glucose; effects of phlorizin. Values are mean ± S.E. of mean. No. of tubules in each series is given in Table 2. In all figures ■, Phlorizin; □, no phlorizin.

and the concentration in the collected fluid was also significantly greater than zero (Table 2; $P < 0.001$). Unexpectedly, in contrast to the findings in the proximal tubule, addition of phlorizin to a non-glucose-containing perfusate (Series D) increased both the secretion of glucose to 11.4 ± 0.8 p-mole min^{-1} (Series C *vs.* D; $P < 0.02$) and the concentration of glucose in collected fluid (Table 2; $P < 0.05$). Thus, there appear to

TABLE 2. Effect of perfusing short loops of Henle in presence and absence of glucose (5 or 0 mM) and presence and absence of phlorizin

					Concentration in collected fluid				
Series	n	Perfusion rate	Inulin ratio	% recollection	Na (mM)	K (mM)	Glucose (mM)	Osmolality (m-osmole kg^{-1} water)	'Deficit' (m-osmole kg^{-1} water)
A Glucose	24	25·1	1·35	100·0	101	3·8	2·96	250	53
		±0·2	±0·02	±1·7	±5	±0·2	±0·21	±4	±9
B Glucose	25	26·2	1·23	100·5	106	2·7	5·59	253	45
+phlorizin		±0·3	±0·02	±2·1	±3	±0·2	±0·10	±5	±7
C No glucose	26	26·3	1·24	96·6	105	3·1	0·39	257	58
		±1·7	±0·02	±2·2	±3	±0·2	±0·05	±4	±5
D No glucose	26	26·5	1·23	98·2	101	3·2	0·54	267	73
+phlorizin		±1·1	±0·01	±2·1	±4	±0·2	±0·03	±3	±7

n = number of tubules perfused.
The osmotically active deficit concentration ('deficit') was calculated as Osmolality − (1·86[Na + K] + [glucose]).
Inulin ratio = In_i/In_o.

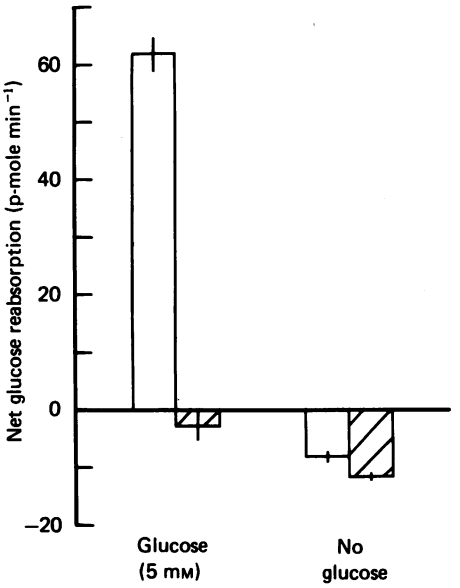

Fig. 2. Net glucose reabsorption in short loops of Henle perfused with fluid containing 5 or 0 mM-glucose; effects of phlorizin. Values are means ± S.E. of mean. No. of tubules in each series is given in Table 2.

Fig. 3. Net sodium reabsorption in short loops of Henle perfused with fluid containing 5 or 0 mM-glucose; effects of phlorizin. Values are means ± S.E. of mean. No. of tubules in each series is given in Table 2.

be important differences in the behaviour of glucose transport in the loop of Henle when compared with the proximal tubule.

In all series, the sodium concentration in collected fluid was much less than that in the perfusate (compare Tables 1 and 2; $P \ll 0{\cdot}001$ in all cases). Although differences in the sodium concentration of collected fluid between the series were not significant,

there were significant differences in the net reabsorption of sodium (Fig. 3). As compared with the glucose series (A), addition of phlorizin (Series B) reduced net sodium reabsorption ($P < 0{\cdot}05$) as did omission of glucose (Series C; $P < 0{\cdot}02$). The addition of phlorizin in the perfusate without glucose (Series D), however, caused a rise in sodium reabsorption compared with Series C ($P < 0{\cdot}05$); sodium reabsorption in Series A and D were not significantly different.

The osmolality of the collected fluid was similar in Series A, B and C, but was greater in Series D where the perfusate was glucose-free but contained phlorizin (Table

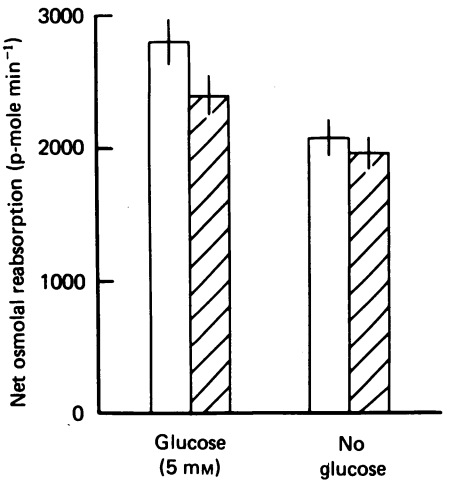

Fig. 4. Net osmolal reabsorption in short loops of Henle perfused with fluid containing 5 or 0 mM-glucose; effects of phlorizin. Values are means ± S.E. of mean. No. of tubules in each series is given in Table 2.

2; $P < 0{\cdot}05$ in all cases). In all cases, the osmolality of the collected fluid was considerably less than that of perfused fluid (compare Tables 1 and 2). The net osmolal flux (Fig. 4) was higher when glucose was present than when it was absent, whether or not phlorizin was present (phlorizin absent, Series A *vs.* C, $P < 0{\cdot}005$; phlorizin present, Series B *vs.* D, $P < 0{\cdot}05$). Any changes caused by addition of phlorizin (Series A *vs.* B and C *vs.* D) were not statistically significant.

The calculated osmotic effects of the solutes measured in the collected fluid were substantially less than its total measured osmolality. This 'deficit' was presumably caused by the movement of some substance into the tubule; the net 'deficit' fluxes were considerable, amounting to at least 600 p-osmole min^{-1} of osmotically active solute (see Fig. 5). The 'deficit' concentration in tubules perfused with glucose-free, phlorizin-containing solution (Series D) was significantly higher than in all other series (Table 2; $P < 0{\cdot}05$ in all cases); there were no significant differences between Series A, B and C. The secretion of osmotically active solute was not significantly altered by the presence of glucose (Series A *vs.* Series C) nor did phlorizin alter the secretion of glucose-containing perfusates (Series A *vs.* B); however, addition of phlorizin to a glucose free solution did cause a marked increase in the net secretion of osmotically active solute (Series D *vs.* Series C; $P < 0{\cdot}02$).

DISCUSSION

The main findings in the present study are *first*, that glucose is reabsorbed significantly by the short loops of Henle (Fig. 2); *secondly*, that intraluminal glucose is associated with increased net sodium and water reabsorption, and phlorizin abolishes these effects of glucose (Figs. 1 and 3); *thirdly*, that phlorizin increases sodium reabsorption from (Fig. 3), and glucose secretion into (Fig. 2), an initially glucose-free perfusate. In addition, secretion of some unknown osmotically active solute ('osmotic deficit', see below) may be enhanced by phlorizin in a glucose-free

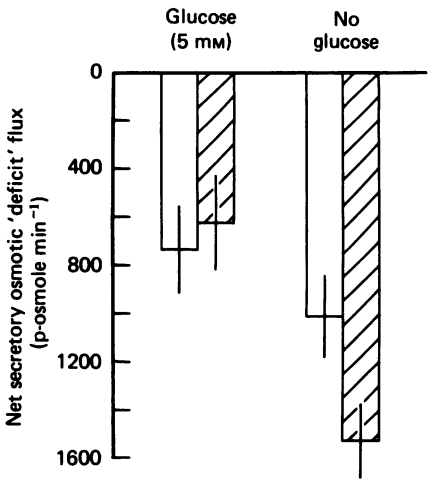

Fig. 5. Net secretory osmotic 'deficit' flux in short loops of Henle perfused with fluid containing 5 or 0 mM-glucose; effects of phlorizin. Osmotic 'deficit flux' is calculated from the osmotic deficit concentration = osmolality − (1·86[Na + K] + [glucose]). Values are means ± S.E. of mean. No. of tubules in each series is given in Table 2.

perfusate (Fig. 5). (Secretion is used throughout this paper to indicate net addition of solute to the tubular fluid, without any implication about the type of mechanism involved).

The short loop of Henle, as perfused in this study, consists of several anatomical segments which have marked functional differences; these include the pars recta of the convoluted tubule, the thin descending limb, the thin ascending limb, the thick ascending limb and the first part of the distal tubule (these latter two segments are suggested to be a single functional entity (Burg, 1976), termed the diluting segment). Several investigators (Morgan & Berliner, 1968; deRouffignac & Morel, 1969; Kokko, 1970; Burg & Green, 1973; Jamison, Buerkert & Lacy, 1973; Rocha & Kokko, 1973; Imai & Kokko, 1974) have demonstrated that these segments have different transport properties with regard to water, ions and solutes such as urea, and it may be that glucose and phlorizin have multiple sites of action in the loop of Henle. Any conclusions that are drawn below must, therefore, be treated with appropriate caution.

Glucose

Glucose can obviously be absorbed from the short loop of Henle, although the rate ($\simeq$ 60 p-mole min^{-1} for whole loop of Henle) is less than in the proximal convoluted tubule ($\simeq$ 200 p-mole min^{-1} for whole proximal tubule during saline perfusion; see Bishop *et al.* 1979). Exact comparisons are difficult because of the inhomogeneity of the loop of Henle, the lack of information about the relative length of its component parts, and uncertainty about whether glucose reabsorption occurs in all its parts. Glucose reabsorption in these studies is unlikely to be due to passive diffusion since (*a*) the perfused concentration was similar to that in plasma (see Table 1) and (*b*) glucose transport was inhibited by phlorizin (Fig. 2), which suggests a carrier mechanism similar to that described for proximal tubule (see review by Lotspeitch, 1960).

When glucose was initially absent from the loop perfusate, secretion occurred. Evidence has been presented previously (Bishop *et al.* 1978*b*) that a similar phenomenon in the proximal tubule is due to a reversal of the carrier, where it is inhibited by phlorizin; but the present study, which shows an *increased* glucose secretion on adding phlorizin, indicates that in the loop of Henle other mechanisms must be operative. There are two possible explanations for this effect of phlorizin in the loop: (*a*) glucose entering at a proximal site in the loop, either by diffusion or a reversal of the carrier, could be removed at a more distal site, the removal being inhibited by phlorizin; this would imply a glucose reabsorption in more distal parts of the loop which is not compatible with von Baeyer's (1975) conclusion that glucose reabsorption in the loop occurred only in the pars recta; (*b*) phlorizin could increase the glucose entry mechanism, either by *stimulating* a reversed glucose carrier (which seems unlikely), or by increasing glucose permeability.

The present data cannot discriminate between these possibilities nor locate the site(s) of glucose reabsorption within the loop. The observation remains, however, that glucose can be reabsorbed by the short loop of Henle.

Interaction of glucose with sodium and water

The effects of glucose on water and ion transport are complex. Net water and sodium fluxes were enhanced by the presence of glucose (compare Series A and C; Figs. 1 and 3) and the effects were abolished by phlorizin (compare Series A and B; Figs. 1 and 3).

One explanation for the effects of glucose would be that it increases net sodium transport in the loop of Henle (either by increasing transport out of, or inhibiting secretion into, the loop) and that this directly or indirectly enhances water removal. The sites where these effects might occur cannot be determined with the present data: but it would seem more likely that effects on water transport occur in the descending part of the loop (including the pars recta) since there is abundant evidence that water permeability is relatively high in the descending limb (Morgan & Berliner, 1968; deRouffignac & Morel, 1969; Jamison *et al.* 1973; Kokko, 1970), while in the ascending thin limb the permeability is much less (Morgan & Berliner, 1968; Imai & Kokko, 1974) and in the diluting segment, water transport is essentially absent (Rocha & Kokko, 1973; Burg & Green, 1973). Sodium movement occurs in all parts of the loop

of Henle, entering the lumen in the descending limb of the loop of Henle (Morgan & Berliner, 1968; Jamison, 1968; deRouffignac & Morel, 1969), leaving the tubule in the thin ascending limb (either passively (Imai & Kokko, 1974) or by a weak transport system (Jamison, 1968; Morgan, 1972; Marsh & Azen, 1975)) and passively following the actively transported chloride in the diluting segment (Rocha & Kokko, 1973; Burg & Green, 1973). If the effects of glucose on sodium reabsorption are linked to the reabsorption of water, then the effects are most likely to be in the descending limb; but such linkage of sodium and water transport in the loop is not established by the present data. The molecular mechanism whereby sodium and glucose are linked in the loop is not known but it cannot be similar to the previously postulated 1:1 stochiometric relationship in the proximal tubule (see review by Silverman, 1976) since the net increased reabsorption of glucose is approximately 60 p-mole compared with over 300 p-mole of sodium (compare Series A and C; Figs. 2 and 3).

Effects of phlorizin

With the dosage used in these experiments, addition of phlorizin to the glucose-containing perfusate completely abolished net transport of glucose by the loop (Fig. 2) and also inhibited the net flux of sodium (Fig. 3) and water (Fig. 1). These results are comparable to those obtained in proximal convoluted tubule (Bishop *et al.* 1978*b*), where the action on ions and water was not considered to be directly related to its action on glucose transport. When phlorizin was added to glucose-free solutions however, the results were unexpected and not predictable from previous proximal tubular results. Thus, glucose secretion was increased as discussed above, and sodium reabsorption was enhanced (Fig. 3) without a concomitant increase in water reabsorption (Fig. 1). The site of such a dissociation between actions on sodium and water is most likely to be the thick ascending limb of the loop of Henle or the diluting segment (Burg, 1976) where water and sodium reabsorption are dissociated (see above); but the mechanism – whether an effect on cation permeability or an indirect effect through altered anion permeability or some other unknown effect – cannot be determined from these experiments. In this respect, effects of phloretin, the aglycone of phlorizin, have been described on cation permeability of lipid bilayers (Anderson, Finkelstein, Katz & Cass, 1976; Melnik, Latorre, Hall & Tosteson, 1977) and red cell membranes (Jennings & Solomon, 1976); and both phlorizin and phloretin have been shown to alter anion permeability in red cell membranes (Gerlach, Deuticke & Duhm, 1964; Schnell, 1972; Passow & Wood, 1974).

Osmotic deficit

The fact that the osmotic effects of the solutes measured in the collected fluid cannot account for the total measured osmolality is hardly surprising since urea is known to enter the loop of Henle (deRouffignac & Morel, 1969; Jamison *et al.* 1973; Imai & Kokko, 1974); but the amount of osmotically active solute added (approx. 20% or more of the total osmolality; Table 2) is high, indicating net secretion of at least 600 p-mole min^{-1} in each series of perfusion (Fig. 5).

The validity of such 'deficit' calculations needs to be carefully examined. When similar calculations (see Methods) were performed on the perfusion solutions, the 'deficit' was not significantly different from zero ($5{\cdot}3 \pm 3{\cdot}1$ m-osmole kg^{-1} water for

all observations); but it was more marked for plasma (16·0 ± 3·9 m-osmole kg^{-1} water for all observations) where other constituents (e.g. Mg^{2+}, organic solutes) might be expected to contribute towards the larger difference. 1·86 is chosen as the osmotic coefficient for the major ionic solutes since this holds for a pure solution of NaCl at this ionic concentration (Robinson & Stokes, 1959); osmotic coefficients in mixtures have not been determined. Using an osmotic coefficient of 2 instead of 1·86, however, does not alter the conclusions although, of course, the absolute magnitude of the flux is reduced. Even accepting the approximate nature of the calculations, the magnitude of the deficit reinforces the conclusion that substantial secretion of osmotically active solute(s) occurred. It seems unlikely that any substance other than urea could account for the major part of the osmotic deficit of some 50 m-osmole kg^{-1} water in any of the collected fluids, although other solutes may have made a minor contribution.

If this deficit is indeed accounted for by urea, then phlorizin also had an effect on urea transport (increased secretion) in the loop of Henle when no glucose was initially present, although there was no effect in glucose-containing perfusates, nor any significant effect of glucose *per se* (Fig. 5). Although the interrelationships between water and urea and between sodium and urea are undoubtedly complex, one possible action of phlorizin might be to enhance tubular permeability to urea (as it may enhance permeability to glucose, see above), a possibility cited as an action of phloretin on red cell membranes (Owen, Steggell & Eyring, 1974).

In conclusion, the short loops of Henle possess a capacity to absorb glucose from glucose-containing perfusates, which is inhibited by phlorizin, and an ability to secrete glucose into an initially glucose-free perfusion, which is enhanced by phlorizin, possibly by increasing the glucose permeability of some segment of the loop. Glucose stimulates net water and sodium reabsorption, probably by an effect on the descending limb of the loop and not a direct 1:1 stimulation of sodium reabsorption; this effect is inhibited by phlorizin. Phlorizin, however, also has additional actions in a glucose-free perfusate, increasing sodium reabsorption and secretion of some osmotically active solute (probably urea) but without an effect on net fluid flux, presumably by a direct or indirect effect on the permeability of the ascending limb of the loop of Henle to such solute. The molecular bases for these actions still require elucidation.

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REFERENCES

ANDERSON, O. S., FINKELSON, A., KATZ, I. & CASS, A. (1976). Effect of phloretin on the permeability of thin lipid membranes. *J. gen. Physiol.* **67**, 749–771.

BISHOP, J. H. V., ELEGBE, R., GREEN, R. & THOMAS, S. (1978*a*). Effects of phlorizin on glucose, water and sodium handling by the rat kidney. *J. Physiol.* **275**, 467–480.

BISHOP, J. H. V. & GREEN, R. (1979*a*). Effect of pregnancy on glucose handling by distal segments of the rat nephron. *J. Physiol.* **289**, 74*P*.

BISHOP, J. H. V. & GREEN, R. (1979*b*). Effects of pregnancy on glucose handling by short loops of Henle in the rat. *J. Physiol.* **296**, 91*P*.

BISHOP, J. H. V., GREEN, R. & THOMAS, S. (1978*b*). Effects of glucose on water and sodium reabsorption in the proximal convoluted tubule of rat kidney. *J. Physiol.* **275**, 481–493.

BISHOP, J. H. V., GREEN, R. & THOMAS, S. (1979). Free flow reabsorption of glucose, sodium osmoles and water in rat proximal convoluted tubule. *J. Physiol.* **288**, 331–351.

BURG, M. B. (1976). Tubular chloride transport and the mode of action of some diuretics. *Kidney Int.* **9**, 189–197.

BURG, M. B. & GREEN, N. (1973). Function of the thick ascending limb of Henle's loop. *Am. J. Physiol.* **224**, 659–668.

FRASCH, W., FRÖHNERT, P. P., BODE, F., BAUMANN, K. & KINNE, R. (1970). Competitive inhibition of phlorizin binding by D-glucose and the influence of sodium; a study on isolated brush border membrane of rat kidney. *Pflügers Arch.* **320**, 265–284.

FRÖHNERT, P. P., HÖHMANN, B., ZWIEBEL, R. & BAUMANN, K. (1970). Free flow micropuncture studies of glucose in the rat nephron. *Pflügers Arch.* **315**, 66–85.

GERLACH, E., DEUTICKE, B. & DUHM, J. (1964). Phosphatpermeabilität und Phosphatstoffwechsel monschiler Erythrocyten und Möglichkeiten ihrer experimentallen Besinflussing, *Pflügers Arch.* **280**, 243–274.

IMAI, M. & KOKKO, J. P. (1974). Sodium chloride urea and water transport in the thin ascending limb of Henle. *J. clin. Invest.* **53**, 393–402.

JAMISON, R. L. (1968). Micropuncture study of segments of thin loop of Henle in the rat. *Am. J. Physiol.* **215**, 236–242.

JAMISON, R. L., BUERKERT, J. & LACY, F. (1973). A micropuncture study of Henle's thin loop in Brattleboro rats. *Am. J. Physiol.* **224**, 180–185.

JENNINGS, M. L. & SOLOMON, A. K. (1976). Interaction between phloretin and the red blood cell membrane. *J. gen. Physiol.* **67**, 381–397.

KOKKO, J. P. (1970). Sodium chloride and water transport in the descending limb of Henle. *J. clin. Invest.* **49**, 1838–1846.

LOTSPEICH, W. D. (1960). Phlorizin and the cellular transport of glucose. *Harvey Lect.* **56**, 63–91.

MARSH, D. J. & AZEN, S. I. (1975). Mechanism of NaCl reabsorption by hamster thin ascending limbs of Henle's loop. *Am. J. Physiol.* **228**, 71–79.

MELNIK, E., LATORRE, R., HALL, J. E. & TOSTESON, D. C. (1977). Phloretin-induced changes in ion transport across lipid bilayer membranes. *J. gen. Physiol.* **69**, 243–257.

MORGAN, T. (1972). Permeability of the thin limbs of the loop of Henle. *Proc. 5th int. Congr. Nephrol., Mexico*, vol. 2, pp. 105–111. Basel: Karger.

MORGAN, T. & BERLINER, R. W. (1968). Permeability of the loop of Henle, vasa recta, and collecting duct to water, urea and sodium. *Am. J. Physiol.* **215**, 108–115.

OWEN, J. D., STEGGALL, M. & EYRING, E. M. (1974). The effect of phloretin on red cell non-electrolyte permeability. *J. Membrane Biol.* **19**, 79–92.

PASSOW, H. & WOOD, P. G. (1974). Current concepts of the mechanism of anion permeability. In *Drugs and Transport Processes*, ed. CALLINGHAM, B. A., pp. 149–171. London: Macmillan.

ROCHA, A. S. & KOKKO, J. P. (1973). Sodium chloride and water transport in the medullary thick ascending limb of Henle. *J. clin. Invest.* **52**, 612–623.

ROBINSON, R. A. & STOKES, R. H. (1959). *Electrolyte Solutions.* 2nd ed., p. 483. London: Butterworths.

ROUFFIGNAC, C. DE & MOREL, F. F. (1969). Micropuncture study of water, electrolytes, and urea movements along the loops of Henle in *Psammomys*. *J. clin. Invest.* **48**, 474–486.

SCHNELL, K. F. (1972). On the mechanism of inhibition of the sulfate transfer across the human erythrocyte membrane. *Biochim. biophys. Acta* **282**, 265–276.

SILVERMAN, M. (1976). Glucose transport in the kidney. *Biochim. biophys. Acta* **457**, 303–307.

TUNE, B. M. & BURG, M. B. (1971). Glucose transport by proximal renal tubule. *Am. J. Physiol.* **221**, 580–585.

VON BAEYER, M. (1975). Glucose transport in the short loop of Henle of the rat kidney. *Pflügers Arch.* **359**, 317–323.