J. Physiol. (1979). **288**, *pp.* 331–351
With 9 text-figures
Printed in Great Britain

FREE-FLOW REABSORPTION OF GLUCOSE, SODIUM, OSMOLES AND WATER IN RAT PROXIMAL CONVOLUTED TUBULE

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(*Received 22 August 1978*)

SUMMARY

1. Reabsorption of glucose, sodium, total solute (osmoles) and water in the rat proximal tubule (pars convoluta) were studied by free-flow micropuncture at normal (saline-infused), suppressed (saline with phlorizin) and elevated (glucose infusion) glucose reabsorption rates.

2. Phlorizin completely inhibited net glucose reabsorption, approximately halved reabsorption of sodium, total solutes and water, and reduced single nephron glomerular filtration rate (*SNGFR*).

3. In saline and glucose-infused groups, there were no significant differences between *SNGFR* nor between reabsorptions (fractional and absolute) of either sodium, total solute or water, which were uniformly distributed along segments assessible to micropuncture.

4. Glucose reabsorptive capacity existed along the entire pars convoluta, with highest reabsorptive rates in convolutions closest to the glomerulus (in saline-infused rats, 90% fractional reabsorption at 2 mm, over 95% at end pars convoluta; in glucose-infused rats, 55 and 90%, respectively).

5. In saline and glucose-infused rats, a significant correlation existed between net glucose and sodium reabsorption, but the regression slopes differed and correlations became non-significant when the reabsorptive fluxes were factored by *SNGFR*.

6. For all groups, the majority of tubular fluid (*TF*) concentrations of osmoles and sodium were lower than those in plasma (over-all mean $TF_{osm}/P_{osm} = 0{\cdot}973 \pm 0{\cdot}004$, $P < 0{\cdot}001$; $TF_{Na}/P_{Na} = 0{\cdot}964 \pm 0{\cdot}005$, $P < 0{\cdot}001$).

7. Correspondingly, calculated osmolal and sodium concentrations in the reabsorbate were greater than those in plasma, and were significantly correlated with distance to puncture site with maximal values in the most proximal convolutions (for osmolality, approximately +70 m-osmole kg^{-1} water at 1 mm).

INTRODUCTION

Since the original proposal by Crane (1962) that glucose transport in the intestine is coupled to active sodium transport, sodium-glucose interactions have been demonstrated in a variety of tissues and organs. For the kidney, data from studies on the whole organ and on proximal tubules provide convincing evidence that the presence of sodium is essential for normal tubular reabsorption of glucose (see reviews by Ullrich, 1976; Silverman, 1976).

In contrast, the limited evidence as to whether primary changes in proximal tubular reabsorption of glucose significantly influence reabsorption of sodium (and hence of water) is conflicting. Although there is an undisputed effect of glucose on electrical potential differences across the proximal tubule (Kokko, 1973; Frömter & Gessner, 1974; Cardinal, Lutz, Burg & Orloff, 1975), it is not certain that this results from direct stimulation of sodium transport (Cardinal *et al.* 1975); and reports of the influence of glucose on fluid reabsorption have ranged from no significant effect (Green & Giebisch, 1975; Bishop, Green & Thomas, 1978*b*) to a 30% stimulation (Weinman, Suki & Eknoyan, 1976).

In a recent study (Bishop *et al.* 1978*b*), a direct examination of this problem was attempted by simultaneous determination of water, sodium and glucose fluxes in micro-perfused middle and late segments of the proximal tubule of rats, using perfusates of varying composition (with and without glucose and phlorizin) in order to manipulate the net glucose flux. The main conclusion was that absence of glucose from the perfusate was associated with only a small (at most 10% and statistically non-significant) reduction in fluid reabsorption. Furthermore, data obtained in this study (Bishop *et al.* 1978*b*) showed several features which were unexpected on the basis of conventional descriptions of proximal tubular function. First, phlorizin, usually regarded as a specific inhibitor of glucose transport (Frasch, Fröhnert, Bode, Baumann & Kinne, 1970) also inhibited fluid reabsorption, both in the presence and absence of glucose in the perfusate. Secondly, for all perfusates, the sodium and osmolal concentrations in the collected fluid tended to be reduced (i.e. the calculated concentrations in the reabsorbate were higher than in perfusates). Pooling the data for all groups, the difference between the osmolalities of reabsorbate and perfusate ($+20{\cdot}0 \pm 9{\cdot}0$ (S.E. of mean) m-osmole kg^{-1} water) was significantly different from zero. This observation conflicted with the conventional view that proximal tubular reabsorption is iso-osmolal (Gottschalk, 1961).

The present study, using classical free-flow micropuncture, has been performed for two main purposes. First, we have repeated the main design of our previous study (Bishop *et al.* 1978*b*) (simultaneous determination of solute and water fluxes in circumstances of primary changes in proximal tubular transport of glucose) but in the more physiological condition of perfusion of the tubule with its own glomerular filtrate. Secondly, we have used this protocol in order to re-examine the experimental basis for current views on proximal tubular fluid reabsorption.

To these ends, experiments were performed in which proximal tubular transport of glucose was manipulated by increasing the filtered load (glucose infusion) or by inhibiting reabsorption (with phlorizin); and free-flow micropuncture collections were used to determine changes in intraluminal solute and osmolal concentrations, to calculate net glucose, sodium, osmolal and water fluxes and to calculate the solute concentrations in the reabsorbate.

METHODS

All experiments were performed on male Sprague-Dawley rats (weighing 140–200 g) which had been allowed free access to a rat pellet diet until 16 h before the experiment and to water until the experiment began. Rats were anaesthetized by an I.P. injection of Inactin (5-ethyl-5-(1-methyl-propyl)-2-thiobarbiturate; Promonta Corp., Hamburg, 120 mg kg^{-1} body weight),

placed on a thermostatically controlled heated table (set to maintain body temperature at 37 °C) and prepared for micropuncture as described previously (Bishop *et al.* 1978*b*).

Three series of experiments were performed on rats infused with: *group I*, 5% glucose (containing [^{3}H]inulin, 5 μc ml.$^{-1}$) at 100 μl. min^{-1} after a priming dose of 1 ml. 0·9% NaCl (containing [^{3}H]inulin, 20 μc ml.$^{-1}$); *group II*, 0·9% NaCl at 200 μl. min^{-1} after a priming dose of 1 ml. 0·9% NaCl (solutions containing [^{3}H]inulin as for group I); and *group III*, 0·9% NaCl as for series 2 but also containing phlorizin (K & K Rare Chemical, Liverpool), 0·1 μmole kg^{-1} body weight, after a priming dose of 1 ml. 0·9% NaCl (containing phlorizin, 2 μmole kg^{-1} body weight). This has been found to give 30% inhibition of glucose reabsorption in the whole kidney (Bishop, Elegbe, Green & Thomas, 1978*a*).

In all these groups, a 1 hour equilibration period was allowed before samples were collected.

Collections from randomly selected proximal tubules were made using pipettes of 10–12 μm tip diameter. A bolus of castor oil B.P. (stained with Sudan Black) with a length at least 5 × tubular diameter was injected, a small negative pressure applied to overcome surface tension at the tip of the pipette and collection initiated. Pressure applied to the pipette was varied to keep the position of the distally placed oil block constant. After fluid collection for 3–7 min, the pipette was rapidly withdrawn into the oil on the surface of the kidney and a small amount of oil drawn in to prevent evaporation of the collected fluid. The tubule was drawn for later reference. Blood samples (100 μl.) were collected from a tail vein into heparinized tubes throughout the experiment and at its termination.

At the end of the experiment, the punctured tubules were reidentified and filled with Microfil (Canton Biomedical Products Inc., Boulder, Colorado). The kidney was removed and stored overnight in deionized water at 4 °C. Next day, following partial digestion of the kidney in 20% NaOH for 25–30 min, the silicone rubber casts were dissected out and drawn using a camera lucida attachment to a stereomicroscope. Lengths were measured using a curvilinear map measurer and converted to original tubular dimensions by comparison with an object micrometer drawn at the same magnification.

The volume of fluid collected from each puncture was measured in a calibrated constant bore capillary of 0·3 mm internal diameter, 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 was compared with that of a similar aliquot of plasma.

For both plasma and tubular fluid, sodium was 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 measured using an enzymatic technique described previously (Bishop *et al.* 1978*b*), reading the fluorescent emission on a fluoromicrophotometer (Aminco Inc., Silver Springs, Maryland).

Calculations

Single nephron glomerular filtration rate, $SNGFR$	$= V\ (TF_{in}/P_{in})$
Nephron filtered load	$SNGFR.P_A$
Fractional excretion	$(TF_A/P_A)/(TF_{in}/P_{in})$
Fractional reabsorption	1 − fractional excretion
Reabsorptive flux of solute, Φ_A	$(SNGFR.P_A)-(V.TF_A)$
Reabsorptive flux of water, Φ_W	$V((TF_{in}/P_{in})-1)$
Reabsorbate concentration of A	Φ_A/Φ_W

where V = volume of fluid collected; TF_{in}/P_{in} = ratio of inulin counts in tubular fluid and plasma; P_A and TF_A = concentrations of A in plasma and tubular fluid, respectively. For P_A values, corrections were not applied for plasma proteins.

Statistical comparisons for mean fluxes between series were performed by t tests. For each experiment, determinations of concentrations in plasma and collected fluid were always performed with standards in the same analytical run. Accordingly, within any one series, comparisons between variables were assessed by the paired t test.

Presentation of data

(*a*) To allow for variation in plasma concentrations (P) in any series, tubular fluid (TF) concentrations are presented as ratios (TF_A/P_A).

(*b*) Data concerning tubular reabsorption of A are presented as: (i) fractional excretion (whence fractional reabsorption = 1 − excretion) and (ii) absolute flux, Φ_A. Variation in fractional reabsorption was less than that in absolute flux, since the former allows for variation in reabsorption which may result from differences in *SNGFR*.

(*c*) In examining a possible correlation between some tubular transport characteristic and the tubular length up to the site of micropuncture, we have used distance from the glomerulus as the most convenient and direct index of the latter.

(*d*) In examining a possible correlation between fractional reabsorption or TF/P ratio and tubular length, statistical analyses were performed using both absolute and log values of the former. For sodium, osmoles and water, higher correlations were almost invariably obtained for absolute values, whereas for glucose, higher correlations were found for log values. Data in Figures and Tables are presented accordingly.

(*e*) Summary data concerning correlations and statistical significance are given in Figure legends. Where r values were not significant, the data were pooled irrespective of puncture site, and mean values calculated.

(*f*) Mean values for Φ_A mm^{-1} length in any one group were derived in two ways: first, by pooling the individual values for each group (data in Table 2) where each individual value represents the mean flux up to the site of micropuncture; and secondly, as the slopes of regression of Φ_A against length (as in Figure legends). For those instances where extrapolation of the calculated regression gives an intercept which differs from zero (i.e. where the data indicate a quantitative difference in function along the length of the tubule), the value for Φ_A mm^{-1} calculated from the slope differs from the mean of the individual values.

To allow for variation in tubular size, alternative indices of length have been used by others: length from glomerulus up to puncture site as fraction of total proximal tubular length (which we discarded because of uncertainty as to where the proximal tubule ended); and fractional fluid reabsorption, which is linearly related to length (see Fig. 1). The latter was discarded since fractional fluid reabsorption is also involved in calculation of solute flux.

It is emphasized that no differences in interpretation arise from the use of these alternative indices of length.

RESULTS

The results of analyses on terminal samples of plasma are summarized in Table 1. There were no significant differences in plasma osmolality between the three groups. However, as compared with the saline controls, glucose infusion was associated with significantly higher plasma glucose and lower sodium concentrations.

Mean single nephron glomerular filtration rates (*SNGFR*) were closely similar in the glucose and saline groups (Table 2), but significantly lower in the saline-phlorizin group. This effect of phlorizin on *SNGFR* in cortical nephrons accessible to micropuncture may be compared with the decrease in whole-kidney glomerular filtration rate previously reported (Bishop *et al.* 1978*a*).

As may be seen in the various Figures, the distance between glomerulus and tubular puncture site varied between 1 and 6 mm in all three groups, the means being closely similar (Table 2).

Water

Data concerning fractional excretion (and hence fractional reabsorption, 1 − fractional excretion) of water are shown in Fig. 1; and calculated values for absolute water reabsorption, Φ_W, are plotted in Fig. 5. For Φ_W, the ranges were essentially similar for the glucose and saline groups (Fig. 5) as were the mean values per unit length (Table 2); for the phlorizin group, maximal values (Fig. 5) and the mean (Table 2) were much lower. For all three groups, the linear correlation between Φ_W

and tubular length to the puncture site was high ($r \geqslant 0{\cdot}60$; $P < 0{\cdot}001$), as was that for fractional excretion ($r \geqslant 0{\cdot}67$; $P < 0{\cdot}001$).

For both fractional excretion (Fig. 1) and Φ_W (Fig. 5), the slopes of the regressions against length of the glucose and saline series were very similar, corresponding to reabsorption of approximately 14 % of filtered water (or 7 nl. min^{-1}) per mm length. In contrast, the slopes for fractional excretion and Φ_W in the phlorizin series were

TABLE 1. Plasma concentrations at termination of experiment (mean ± S.E.)

Group	*n*	Sodium (m-mole $l.^{-1}$)	Glucose (m-mole $l.^{-1}$)	Osmolality (m-osmole kg^{-1} water)
1. Glucose	10	139·9 ± 2·4	10·78 ± 1·31	292 ± 5
2. Saline	11	149·9 ± 3·4	4·01 ± 0·25	299 ± 3
3. Saline-phlorizin	7	144·3 ± 2·1	3·88 ± 0·30	297 ± 3

TABLE 2. Mean (± S.E.) data for all micropuncture sites (*n* = number of samples in each group. Number of animals shown in Table 1)

n	Glucose 56	Saline 40	Saline-phlorizin 46
Distance to site (mm)	3·03 ± 0·14	2·94 ± 0·17	3·00 ± 0·18
SNGFR (nl. min^{-1})	34·1 ± 1·8	34·4 ± 2·4	29·2 ± 1·1*
TF_{osm}/P_{osm}	0·968 ± 0·007 ($P < 0{\cdot}001$)	0·986 ± 0·006 ($P < 0{\cdot}05$)	0·964 ± 0·008* ($P < 0{\cdot}001$)
TF_{Na}/P_{Na}	0·989 ± 0·005*** ($P < 0{\cdot}05$)	0·944 ± 0·008 ($P < 0{\cdot}001$)	0·949 ± 0·004 ($P < 0{\cdot}001$)
Φ_W (nl. min^{-1} mm^{-1})	4·78 ± 0·34	4·65 ± 0·57	2·32 ± 0·14***
Φ_{osm} (p-osmole min^{-1} mm^{-1})	1473 ± 100	1444 ± 175	738 ± 46***
Φ_{Na} (p-mole min^{-1} mm^{-1})	649 ± 44	746 ± 74	403 ± 24***
Φ_G (p-mole min^{-1} mm^{-1})	84·8 ± 6·7***	36·5 ± 2·5	−0·97 ± 1·91***

TF = tubular fluid, *P* = plasma. Φ = net reabsorptive flux (subscripts W = water, osm = osmoles, Na = sodium, G = glucose). Each flux value was calculated as (total net flux)/(distance to site × time).

For the glucose and saline-phlorizin groups, asterisks = level of statistical significance when compared with saline group (*, **, *** = $P < 0{\cdot}05$, $< 0{\cdot}01$ and $< 0{\cdot}001$, respectively).

For TF/P ratios, statistical value under each ratio indicates probability that the ratio = 1·0. Pooling the ratios for all three groups, the over-all mean $TF_{osm}/P_{osm} = 0{\cdot}973 \pm 0{\cdot}004$ ($P < 0{\cdot}001$) and the over-all mean $TF_{Na}/P_{Na} = 0{\cdot}964 \pm 0{\cdot}005$ ($P < 0{\cdot}001$).

much shallower. Because the *SNGFR* in the phlorizin experiments was significantly lower than in the other two series (Table 2), the reduction in Φ_W (to approximately 25 %) was greater than that in fractional water reabsorption (to approximately 40 %).

Glucose

As explained in Methods, correlations between the various aspects of glucose transport and tubular length were higher when the former were expressed as log values. The most substantial changes in glucose reabsorption occurred in the most proximal convolutions. For the saline group, this is even more apparent in that for both TF_G/P_G and fractional excretion (Fig. 2), extrapolation of the regression lines produced intercepts significantly less than 1·0.

In the saline group, the changes in TF_G/P_G (Fig. 2*A*) correspond with a steep fall

from 4 m-mole l.$^{-1}$ in plasma to 0·7 m-mole l.$^{-1}$ at 1 mm, with only a small further fall to 0·3 m-mole l.$^{-1}$ at 5 mm. The data for fractional excretion (Fig. 2*B*) show that 80% of filtered glucose was reabsorbed by the end of the first millimetre, over 90% by 3 mm and over 95% up to 5 mm. Thus the glucose reabsorption at the most distal sites of micropuncture was dominated by the avid reabsorption in the most proximal convolutions, close to the glomerulus.

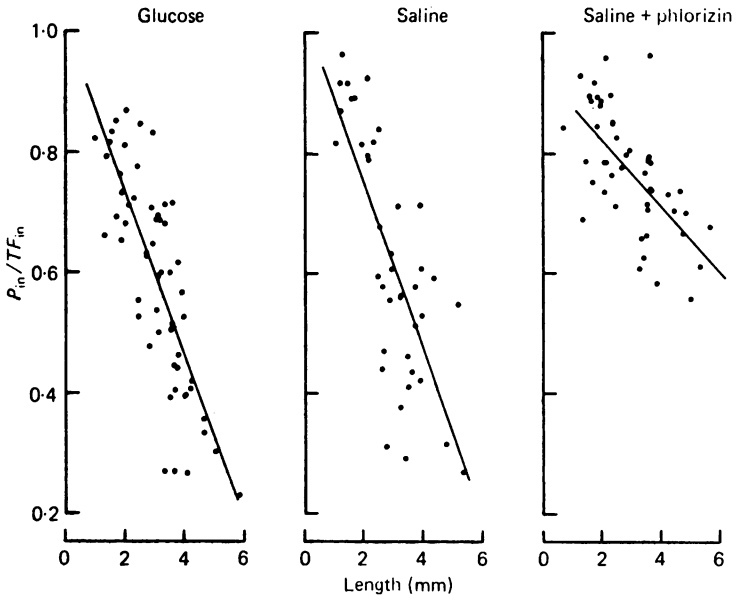

Fig. 1. Relations between fractional excretion of water (P_{in}/TF_{in}) and distance from glomerulus to puncture site in glucose-infused, saline-infused and saline (with phlorizin)-infused groups. In this and subsequent Figures, lines indicate significant regressions; the relevant equations are:

(*i*) glucose, $y = 1{\cdot}02 - 0{\cdot}139x$, $r = -0{\cdot}80$, $P < 0{\cdot}001$;
(*ii*) saline, $y = 1{\cdot}04 - 0{\cdot}140x$, $r = -0{\cdot}75$, $P < 0{\cdot}001$;
(*iii*) saline + phlorizin, $y = 0{\cdot}93 - 0{\cdot}055x$, $r = -0{\cdot}67$, $P < 0{\cdot}001$.

In no case was the intercept on the ordinate significantly different from 1·00. For the saline-phlorizin group, the slope of the regression was significantly different from that for saline ($P < 0{\cdot}001$).

For the glucose-infused group, the change in TF_G/P_G (Fig. 2*A*) correspond with a fall from over 10 m-mole l.$^{-1}$ in the plasma to approximately 8 m-mole l.$^{-1}$ at 1 mm, to 5 m-mole l.$^{-1}$ at 3 mm and to 3 m-mole l.$^{-1}$ at 5 mm. The data for fractional excretion (Fig. 2*B*) show a highly significant correlation between log values and tubular length. The regression line corresponds with reabsorption of approximately 30% of filtered glucose in the first millimetre, over 70% at 3 mm and 90% up to 5 mm. As with the saline group, the highest fractional reabsorption per mm occurred in the most proximal segments.

The range of values for net reabsorptive fluxes, Φ_G, was far wider in the glucose infused group than in the saline-infused rats, particularly for the more distal puncture sites, with maximal values almost four times greater (Fig. 5). Similarly, the mean

Φ_G mm^{-1} was much greater in the glucose group than in the saline-infused rats (Table 2).

For the phlorizin group, the Φ_G data (Fig. 5; Table 2) and the fractional excretion

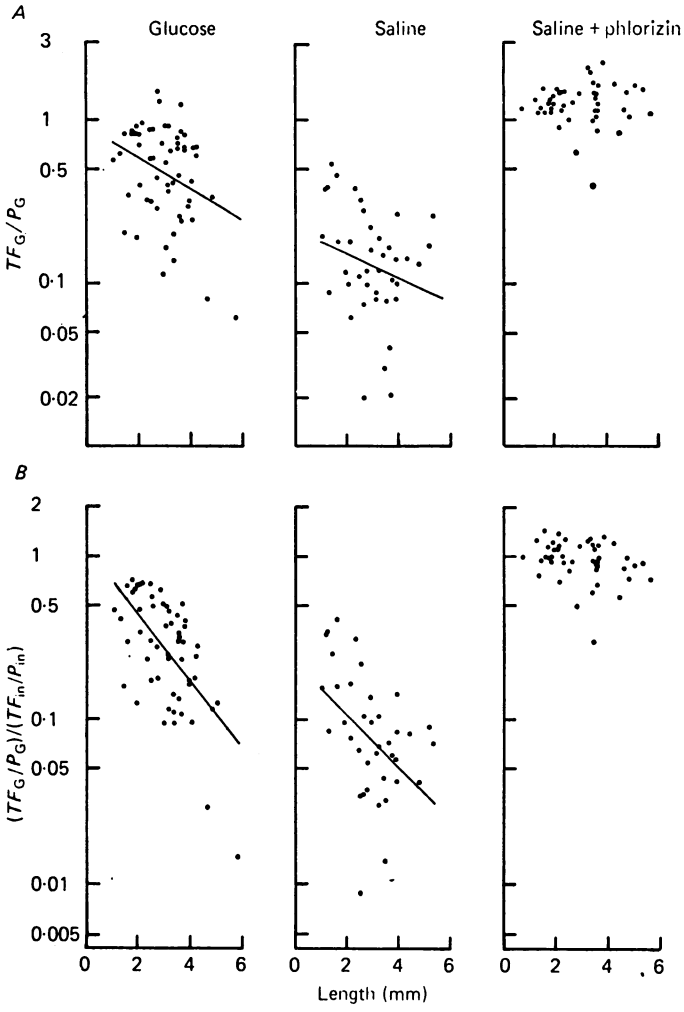

Fig. 2. Relations between distance from glomerulus to puncture site and *A*, tubular fluid/plasma concentration ratio for glucose, TF_G/P_G. *B*, fractional excretion of glucose $(TF_G/P_G)/(TF_{in}/P_{in})$. The equations of the lines of best fit are:

A (*i*) glucose, $\log y = -0{\cdot}041 - 0{\cdot}095x$, $r = -0{\cdot}33$, $P < 0{\cdot}01$;
(*ii*) saline, $\log y = -0{\cdot}674 - 0{\cdot}070x$, $r = -0{\cdot}32$, $P < 0{\cdot}01$;
(*iii*) saline + phlorizin, no significant correlation, $r = -0{\cdot}04$.

The intercept on the ordinate was significantly different from 1·00 for the saline group ($P < 0{\cdot}001$).

B (*i*) glucose, $\log y = 0{\cdot}061 - 0{\cdot}210x$, $r = -0{\cdot}62$, $P < 0{\cdot}001$;
(*ii*) saline, $\log y = -0{\cdot}647 - 0{\cdot}160x$, $r = -0{\cdot}44$, $P < 0{\cdot}005$;
(*iii*) saline + phlorizin, no significant correlation, $r = -0{\cdot}26$.

The intercept on the ordinate was significantly different from 1·00 for the saline group ($P < 0{\cdot}001$).

data (Fig. 2*B*) show that the dose of phlorizin effectively abolished net glucose transport. The mean net fractional reabsorption value for all punctured sites (1·8 ± 3·5 %) did not differ significantly from zero.

Sodium; osmoles

Comparison of Figs. 3*B* and 4*B* with Fig. 1 shows that for all three groups, the changes in the fractions of filtered sodium and total osmoles remaining at the various

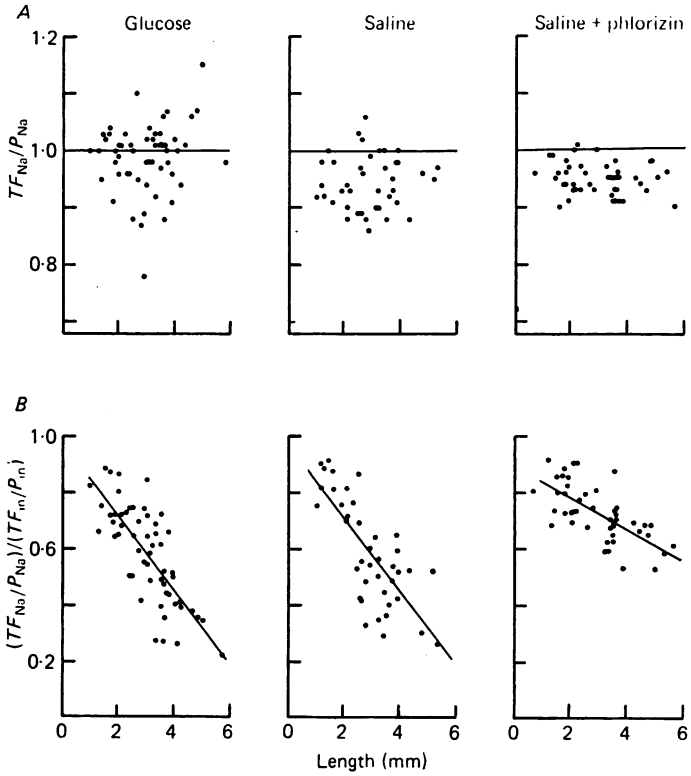

Fig. 3. Relations between distance from glomerulus to puncture site and *A*, tubular fluid/plasma concentration ratio for sodium, TF_{Na}/P_{Na}; *B*, fractional excretion of sodium $(TF_{Na}/P_{Na})/(TF_{in}/P_{in})$. The equations of the lines of best fit are:

A (*i*) glucose, no significant correlation, $r = 0{\cdot}14$;
(*ii*) saline, no significant correlation, $r = 0{\cdot}07$;
(*iii*) saline + phlorizin, no significant correlation, $r = -0{\cdot}21$.

B (*i*) glucose, $y = 0{\cdot}90 - 0{\cdot}135x$, $r = -0{\cdot}80$, $P < 0{\cdot}001$;
(*ii*) saline, $y = 0{\cdot}975 - 0{\cdot}131x$, $r = -0{\cdot}75$, $P < 0{\cdot}001$;
(*iii*) saline + phlorizin, $y = 0{\cdot}895 - 0{\cdot}055x$, $r = -0{\cdot}67$, $P < 0{\cdot}001$.

In no case was the ordinate intercept significantly different from 1·0. For the saline-phlorizin group, the slope of the regression was significantly different from that of the saline group ($P < 0{\cdot}001$).

puncture sites were essentially similar in pattern to the changes described for water. Correlations between fractional excretion (and hence fractional reabsorption) and length were high for both sodium ($r \geqslant 0{\cdot}67$; $P < 0{\cdot}001$) and osmoles ($r \geqslant 0{\cdot}51$; $P < 0{\cdot}001$).

For the glucose and saline groups, there were no significant differences between the regression slopes for either sodium or osmoles, both for fractional (Figs. 3*B* and 4*B*) and for absolute flux (Fig. 5 for sodium) values. Similarly, the ranges of values of both net Φ_{Na} and net Φ_{osm} were similar in these two groups. The similarity of the relationships between fractional sodium and fractional osmolal reabsorption against the tubular length implies a corresponding similarity between sodium and osmolal fluxes in these groups. For convenience, therefore, net Φ_{osm} (approximately double Φ_{Na}) is not plotted in Fig. 5.

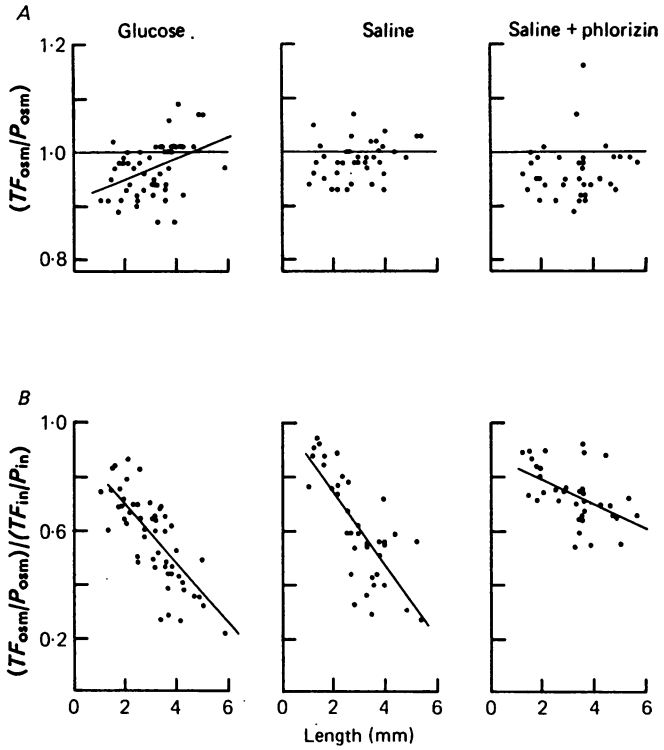

Fig. 4. Relations between distance from glomerulus to puncture site and *A*, tubular fluid/plasma concentration ratio for osmoles, TF_{osm}/P_{osm}; *B*, fractional excretion of osmoles $(TF_{osm}/P_{osm})/(TF_{in}/P_{in})$. The equations of the lines of best fit are:

A (*i*) glucose, $y = 0{\cdot}909 + 0{\cdot}019x$, $r = 0{\cdot}40$, $P < 0{\cdot}01$;
(*ii*) saline, no significant correlation, $r = 0{\cdot}28$;
(*iii*) saline + phlorizin, no significant correlation, $r = 0{\cdot}09$.

For the glucose group, the ordinate intercept was not significantly different from 1·0.

B (*i*) glucose, $y = 0{\cdot}915 - 0{\cdot}110x$, $r = -0{\cdot}74$, $P < 0{\cdot}001$;
(*ii*) saline, $y = 1{\cdot}014 - 0{\cdot}135x$, $r = -0{\cdot}77$, $P < 0{\cdot}001$;
(*iii*) saline + phlorizin, $y = 0{\cdot}884 - 0{\cdot}045x$, $r = -0{\cdot}51$, $P < 0{\cdot}001$.

For the saline + phlorizin group, the ordinate intercept was significantly different from 1·0 ($P < 0{\cdot}001$); and the slope of regression was significantly different from that of the saline group ($P < 0{\cdot}001$).

By 5 mm, approximately two thirds of filtered sodium (Fig. 3*B*) and osmoles (Fig. 4*B*) had been reabsorbed in the glucose and sodium infused animals. In contrast, reabsorption was approximately halved for the phlorizin group. As described above

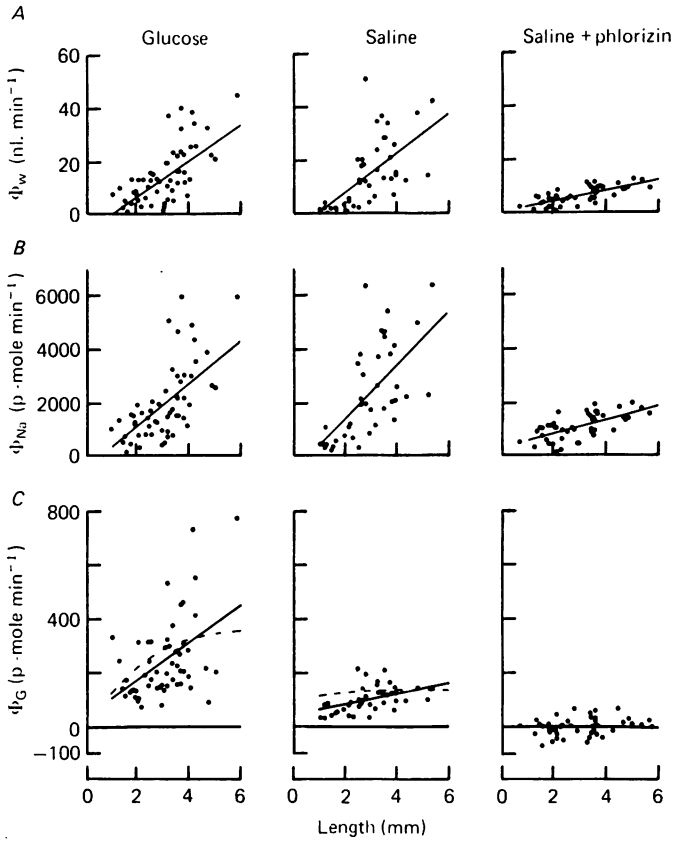

Fig. 5. Relations between distance from glomerulus to puncture site and the net reabsorptive fluxes of water (Φ_W), sodium (Φ_{Na}) and glucose (Φ_G). For Φ_G, the continuous lines indicate significant linear correlations (for glucose $P < 0{\cdot}001$; for saline $P < 0{\cdot}005$); these are considered not to represent the true functional relation with length, which is better represented by the log correlations in Fig. 2. Using the regression lines for fractional glucose excretion (Fig. 2) and mean values for filtered glucose, mean values for Φ_G can be calculated along the proximal tubule: these are indicated as the interrupted lines. The equations of the lines of best fit for other parts of the figure are:

A (*i*) glucose, $y = -6{\cdot}12 + 6{\cdot}94x$, $r = 0{\cdot}66$, $P < 0{\cdot}001$;
(*ii*) saline, $y = -6{\cdot}46 + 7{\cdot}30x$, $r = 0{\cdot}61$, $P < 0{\cdot}001$;
(*iii*) saline + phlorizin, $y = 1{\cdot}41 + 1{\cdot}74x$, $r = 0{\cdot}69$, $P < 0{\cdot}001$.

The ordinate intercept is significantly different from 0 in the glucose group ($P < 0{\cdot}05$) and of borderline significance in the saline group ($0{\cdot}1 > P > 0{\cdot}05$). The slope of the regression for the saline − phlorizin group was significantly different from that of the saline group ($P < 0{\cdot}005$).

B (*i*) glucose, $y = -475 + 810x$, $r = 0{\cdot}58$, $P < 0{\cdot}001$;
(*ii*) saline, $y = -551 + 996x$, $r = 0{\cdot}62$, $P < 0{\cdot}001$;
(*iii*) saline + phlorizin, $y = 354 + 261x$, $r = 0{\cdot}66$, $P < 0{\cdot}001$.

In no case was the intercept on the ordinate significantly different from 0. The slope of the regression for the saline-phlorizin group was significantly different from that of the saline group ($P < 0{\cdot}005$).

for water, the significantly lower *SNGFR* in this group accounts for the fact that the reductions in Φ_{Na} and Φ_{osm} were relatively greater than the reductions in the fractional reabsorptions.

For all three groups, correlations for both TF_{Na}/P_{Na} and TF_{osm}/P_{osm} against length were low and non-significant except for a significant increase with length for TF_{osm}/P_{osm} in the glucose group (Fig. 4*A*), i.e. after the first millimetre, tubular

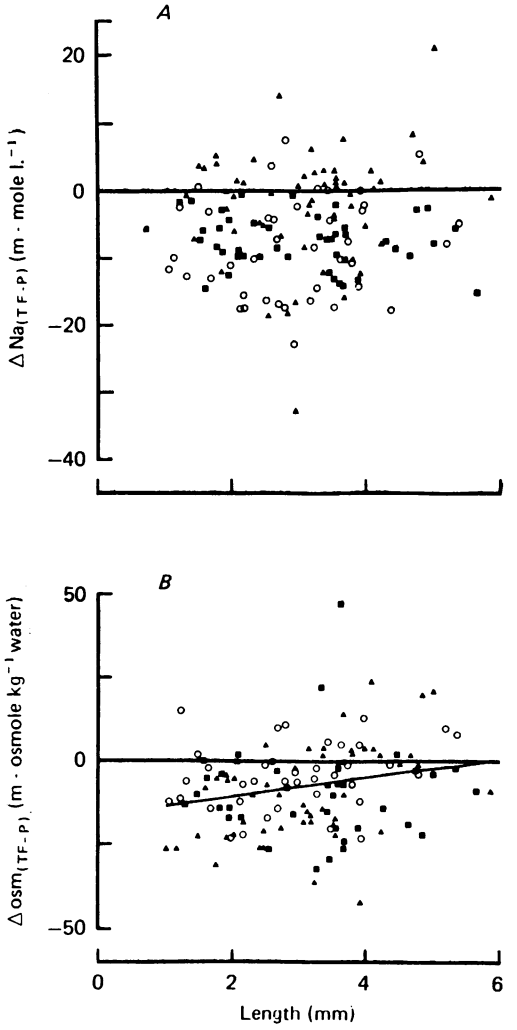

Fig. 6. Pooled data from glucose-infused (▲), saline-infused (○) and saline (with phlorizin)-infused (■) groups, showing relations between distance from glomerulus to puncture site and *A*, difference between plasma and tubular fluid concentrations of sodium (Δ $Na_{(TF-P)}$): no significant correlation, $r = 0{\cdot}051$; *B*, difference between plasma and tubular fluid concentrations of osmoles (Δ $osm_{(TF-P)}$): $y = -15{\cdot}97 + 2{\cdot}66x$, $r = 0{\cdot}22$, $P < 0{\cdot}02$.

sodium and osmolal concentrations showed no consistently significant change relative to plasma. However, Figs. 3*A* and 4*A* clearly show a tendency to ratios below 1·0 for both sodium and osmoles; mean values were significantly less than 1·0 for each

group and for the pooled data from all three groups (Table 2). Although the ratios were not identical for the three groups, we consider that the values were sufficiently similar that (together with the consistently significant direction) pooling is permissible.

This is shown in another manner in Fig. 6, where the absolute differences between tubular and plasma concentrations are plotted against tubular length. For both sodium and osmoles, the mean differences for the pooled data from all three series ($-5{\cdot}10 \pm 0{\cdot}66$ m-mole $l.^{-1}$ and $-7{\cdot}80 \pm 1{\cdot}17$ m-osmole kg^{-1} water, respectively) were significantly different from zero ($P < 0{\cdot}001$ in both cases). The difference in sodium concentration did not correlate significantly with length, whereas the difference in osmolality was significantly related, with the minimal calculated tubular osmolality occurring at 1 mm (or earlier) and a calculated value approaching that of plasma at about 6 mm.

Thus the data indicate a reduction in sodium and osmolal concentrations of the tubular fluid within the first millimetre, i.e. reabsorption of a solution with a higher concentration of sodium and osmoles than either plasma or tubular fluid. This will be examined in Discussion.

DISCUSSION

As will be discussed below, many aspects of the pattern of change in intraluminal fluid correspond with conventional descriptions; but the significantly lower values for osmolal and sodium concentrations in tubular fluid, as compared with plasma, so conflict with such descriptions that a critical scrutiny of experimental and analytical techniques is required.

The micropuncture methods employed here are essentially similar to those used in numerous previous studies, as is the use of labelled inulin for determination of *SNGFR* and fractional water reabsorption (and so for calculation of Φ_W). Values for *SNGFR* are similar to those reported by others (see Table 1 in review by Wright & Giebisch, 1971). The enzymic microdetermination of glucose is sensitive and reproducible; for this laboratory, the 4% coefficient of variation (Bishop *et al.* 1978*b*) is very similar to values (4·5%) reported by others (Fröhnert, Höhmann, Zwiebel & Baumann, 1970; von Baeyer, von Conta, Haeberle & Deetjen, 1973).

In our hands, helium-glow photometry for sodium (Vurek & Bowman, 1965) and cryosmometry for osmolality (Ramsay & Brown, 1955) give complete recoveries and and values not significantly different from those provided by standard macro methods. On standard solutions treated in the same manner as micropuncture samples, the coefficient of variation for sodium is less than 4% (cf. Jamison, 1968; Seely & Dirks, 1969); and for osmolality less than 3% (cf. Jamison, 1968; Persson & Ulfendahl, 1970). Possible observer bias was avoided in the following manner. Micropuncture and subsequent microdissection of puncture site were performed by one of the co-authors; analyses of tubular fluid samples were performed by others (sodium by another co-author; inulin, osmolality and glucose by skilled assistants) who were unaware of the experimental group and of the puncture site.

Changes in volume and composition of tubular fluid

Proximal tubular puncture sites are limited to convolutions (pars convoluta) of superficial nephrons accessible at the renal cortical surface. According to Rector, Brunner, Sellman & Seldin (1966), 95% of punctures of end surface convolutions

correspond to 55–60% of total proximal tubular length in the rat. In the present study, puncture sites varied between 1 and 6 mm from the glomerulus in each group, the majority lying between 1½ and 5 mm. In separate studies (unpublished) performed for other purposes in this laboratory, the mean proximal tubular length in the same strain of rat at comparable weight was 6·70 mm, and for pars convoluta was 5·01 mm (75% of total length). Thus, the majority of sites punctured in the present study range between 20% and 75% of total proximal tubular length, with the most distal sites being close to the end of the pars convoluta.

Water; sodium; osmoles

On this basis, the reabsorption of approximately 70% of filtered water, sodium and osmoles at 5 mm in the saline-infused group corresponds with the general view that in a variety of circumstances some 2/3 to 3/4 of filtered fluid is reabsorbed in the pars convoluta (Gottschalk, 1963; Giebisch & Windhager, 1973). Furthermore, in fluid samples obtained from the most distal puncture sites, osmolal and sodium concentrations were essentially similar to those in plasma (Figs. 3, 4, and 6); in this respect, there were no consistent differences between the three groups. Again, this would conform with the general view that proximal tubular reabsorption is isosmolal with water movement secondary to primary movement of solute, mainly sodium (Gottschalk, 1963; Giebisch & Windhager, 1973).

In contrast, for more proximal convolutions, a large proportion of samples from all three groups provided sodium and osmolal concentrations lower than those in plasma (Figs. 3, 4, and 6) such that the mean TF/P ratios for each group were significantly less than 1·0 (Table 2), and the mean differences between tubular and plasma concentrations for the pooled data for all three groups (Fig. 6) were significant for both sodium and osmoles.

Although most of the earlier micropuncture studies reported TF_{osm}/P_{osm} close to 1·0 'within limits of error', close scrutiny of the data in some more recent works shows a tendency to lower values which seem likely to have approached statistically significant levels, e.g. values of $0{\cdot}98 \pm 0{\cdot}02$ (S.D.) for Rhesus monkey (Bennett, Brenner & Berliner, 1968), $0{\cdot}965 \pm 0{\cdot}004$ (S.E.) for Psammomys (Morel, de Rouffignac, Marsh, Guinnebault & Lechene, 1969) and $0{\cdot}98 \pm 0{\cdot}007$ (S.E.) for rat (Lechene, Morel, Guinnebault & de Rouffignac, 1969). Published data for mean values of TF_{Na}/P_{Na} are more consistently close to 1·0.

For several reasons we consider that the present data have real significance.

(*a*) The accuracy, sensitivity and reproducibility of the ultramicroanalytical techniques are comparable with those reported by others; and we are confident that these are adequate to detect (with statistically significant consistency in large numbers) differences in tubular concentrations of the magnitude reported here.

(*b*) If experimental errors contributed significantly to the lower values, these would be expected to be distributed uniformly along the length of the pars convoluta. For the pooled data, the significant correlation of osmolality with length, with lowest values in the most proximal convolutions (Fig. 6), indicates functional significance.

(*c*) The data are compatible with recent theoretical analyses of proximal tubular reabsorption of fluid (Sackin & Boulpaep, 1975; Andreoli, 1977), and with data from *in situ* microperfusion of proximal tubular segments (Bishop *et al.* 1978*b*).

Composition of reabsorbate

Solute concentrations in proximal tubular reabsorbate may be calculated by dividing solute fluxes by the corresponding water fluxes. In Fig. 7, the differences between calculated reabsorbate concentrations and corresponding plasma concentrations are plotted against tubular length.

For reabsorbate osmolality, the highly significant negative correlation with length corresponds with an osmolality approximately 70 m-osmole kg^{-1} water greater than that of plasma at 1 mm length, declining to approximately iso-osmolal values at 5–6 mm. A similar pattern applies for calculated sodium concentration in reabsorbate (Fig. 7); the absolute values (approximately half the osmolal values) indicate that the changes in osmolality along the pars convoluta are mainly ascribable to changes in sodium and associated anions. For reabsorbate osmolality, the average value (approximately $+35$ m-osmole kg^{-1} water) is compatible with that derived from *in situ* perfusion of mid and distal segments of the pars convoluta ($+20$ m-osmole kg^{-1} water; Bishop *et al.* 1978*b*).

These data provide further support for the conclusion derived from theoretical analysis by Sackin & Boulpaep (1975), that proximal tubular reabsorbate is hyperosmolal to tubular fluid. Similarly, Andreoli (1977) has discussed a model in which a small reduction in luminal osmolality could provide a significant driving force for net fluid reabsorption without requiring the intercellular standing osmotic gradient postulated by Diamond & Bossert (1967).

The present data suggest that maximal reabsorbate hyperosmolality (and minimal luminal osmolality) is generated in convolutions close to the glomerulus. Whether this is attributable to a relatively greater rate of solute reabsorption as compared with later convolutions, or to a relatively lower rate of water movement (due to a lower permeability to water), cannot be determined from these data (although the negative intercepts for water flux (Fig. 5) in the glucose group ($P < 0{\cdot}05$) and saline group ($0{\cdot}1 > P > 0{\cdot}05$) would be compatible with lower water movement). Similarly, the relative contribution of the higher rate of glucose reabsorption in the most proximal convolutions (in the glucose and saline groups) is also uncertain. More precise information on the events of these most proximal convolutions would require direct sampling from the first millimetre.

Glucose

There have been very few micropuncture studies of glucose reabsorption in free-flow circumstances since the classical study of Walker, Bott, Oliver & MacDowell (1941), which formed the basis of the conventional view that at normal plasma glucose concentrations, filtered glucose is almost completely reabsorbed by the end of the proximal tubule. The present value of over 96% reabsorption by the end of the pars convoluta compares with those of 98% for the rat (Fröhnert *et al.* 1970) and over 90% for the dog (Wen, 1976). The data in Fig. 2 confirm that at normal plasma glucose concentrations, the most avid glucose reabsorption occurred close to the glomerulus (Walker *et al.* 1941: Rohde & Deetjen, 1968; Fröhnert *et al.* 1970; Wen, 1976).

The only previous data (Baines, 1971) for elevated plasma glucose concentrations

in any species concern maximal glucose reabsorption rates in single nephrons in the rat. Unfortunately, data were presented for end pars convoluta only. In the present study Fig. 2 shows that at raised plasma glucose levels, a significant quantity of glucose escapes reabsorption in the earliest convolutions and that most of this is reabsorbed in mid and later convolutions. However, the 90% reabsorption value at 5 mm (comparable with 92% at the most distal of accessible proximal tubular

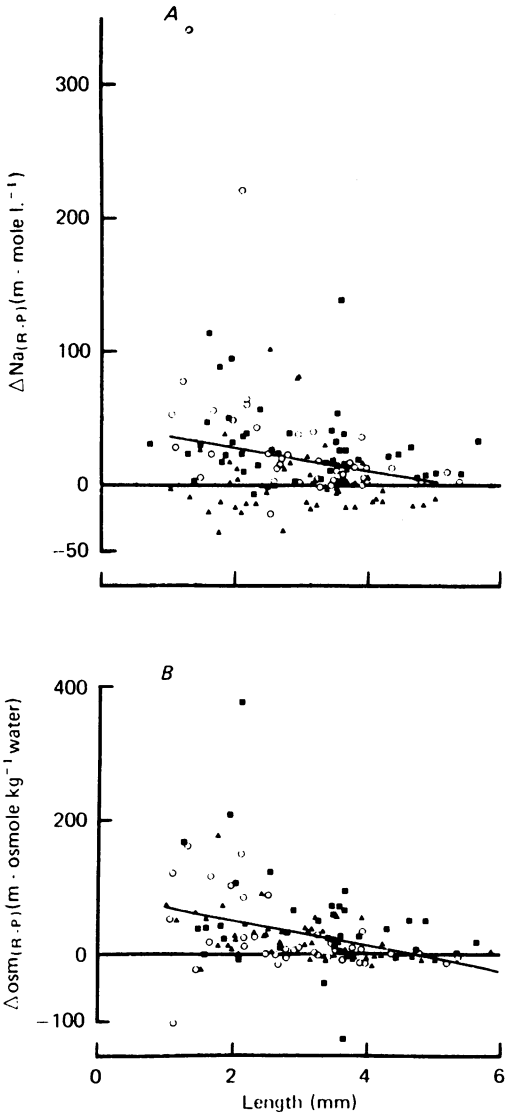

Fig. 7. Pooled data from glucose-infused (▲), saline-infused (○) and saline (with phlorizin)-infused (■) groups, showing relations between distance from glomerulus to puncture site and *A*, difference between calculated reabsorbate and plasma concentrations of sodium ($\triangle$ $Na_{(R-P)}$): $y = 39{\cdot}6 - 7{\cdot}05x$, $r = -0{\cdot}23$, $P < 0{\cdot}01$; ordinate intercept significantly different from 0 ($P < 0{\cdot}001$); *B*, difference between calculated reabsorbate and plasma concentrations of osmoles ($\triangle$ $osm_{(R-P)}$): $y = 86{\cdot}8 - 18{\cdot}49x$, $r = -0{\cdot}35$, $P < 0{\cdot}001$; ordinate intercept significantly different from 0 ($P < 0{\cdot}001$).

convolutions in the study of Baines, 1971) means that glucose reabsorption is less complete at this point than at normal plasma glucose concentrations. It can be deduced from the data in Fig. 2 and Φ_G data in Fig. 5 that at elevated plasma glucose concentrations, the rate of glucose reabsorption in the first mm was unlikely to be dissimilar from that at normal plasma glucose levels, so that the first convolutions are likely to be operating at close to maximal capacity even at normal plasma glucose concentrations; and that even when greater quantities of glucose, at higher glucose concentrations, were delivered to the mid portions of the pars convoluta, glucose reabsorptive rates remained lower than those found in the most proximal convolutions at normal plasma concentrations. This seems to be the best evidence, albeit indirect, that the glucose transporting capacity of convolutions close to the glomerulus is greater than that of more distal portions of the pars convoluta. For the isolated perfused proximal tubule of rabbit, direct evidence exists that the glucose transport capacity of pars convoluta is much greater than that of pars recta (Tune & Burg, 1971).

At normal plasma glucose concentrations, the rapid decrease in intraluminal glucose concentration in the earliest convolutions was followed by only small changes through the mid and late convolutions, where the concentration remained essentially steady at less than 0·5 m-mole $l.^{-1}$, despite the fact that a significant reabsorptive capacity exists in these later convolutions (as shown in the glucose-infusion experiments). Similar equilibrium conditions have been reported previously in free-flow micropuncture experiments (Rohde & Deetjen, 1968; Fröhnert *et al.* 1970) and with *in situ* microperfusions (Stolte, Hare & Boylan, 1972; von Baeyer *et al.* 1973; Bishop *et al.* 1978*b*). As discussed previously (Bishop *et al.* 1978*b*), such steady-state concentrations are predictable on the basis of a definite, though low, passive permeability of the tubule to glucose (Loeschke, Baumann, Renschler & Ullrich, 1969; Stolte *et al.* 1972; von Baeyer *et al.* 1973), and on the basis that the glucose carrier may be reversible (Bishop *et al.* 1978*b*).

Effects of phlorizin

As in our previous study on *in situ* microperfusion of pars convoluta segments (Bishop *et al.* 1978*b*), phlorizin in appropriate dose effectively inhibited net transport of glucose in the present free-flow experiments (Figs. 2, 5) due to its competitive inhibitor action on the glucose carrier (Frasch *et al.* 1970). It might be expected, therefore, that intraluminal glucose concentration would rise substantially in consequence of progressive reabsorption of water along the nephron, as appeared to be the case for the experiments of Walker *et al.* (1941); whereas Fig. 2 shows that a significant increase did not occur in the present study.

Two possible explanations may be suggested. First, the permeability to glucose, although low, may permit some passive reabsorption of glucose as the intralumnal concentration rises. That some reabsorption may have occurred is indicated by the data for fractional reabsorption (Fig. 2) and for Φ_G (Fig. 5) in the phlorizin group, although the correlations with length were non-significant. Secondly, it is evident that phlorizin caused a substantial reduction in net water reabsorption (Figs. 1, 5), so that the consequent increase in intraluminal glucose concentration would be correspondingly reduced.

Renal effects of phlorizin additional to those on glucose transport have been demonstrated previously in studies on the whole kidney (Bishop *et al.* 1978*a*). In the *in situ* perfused proximal tubule, phlorizin reduced net water flux (significantly) and appeared to cause a small (though non-significant) reduction in net sodium flux (Bishop *et al.* 1978*b*). In the present experiments, it is clear that phlorizin caused substantial and significant reductions in reabsorption of both sodium (Figs. 3, 5) and water (Figs. 1, 5).

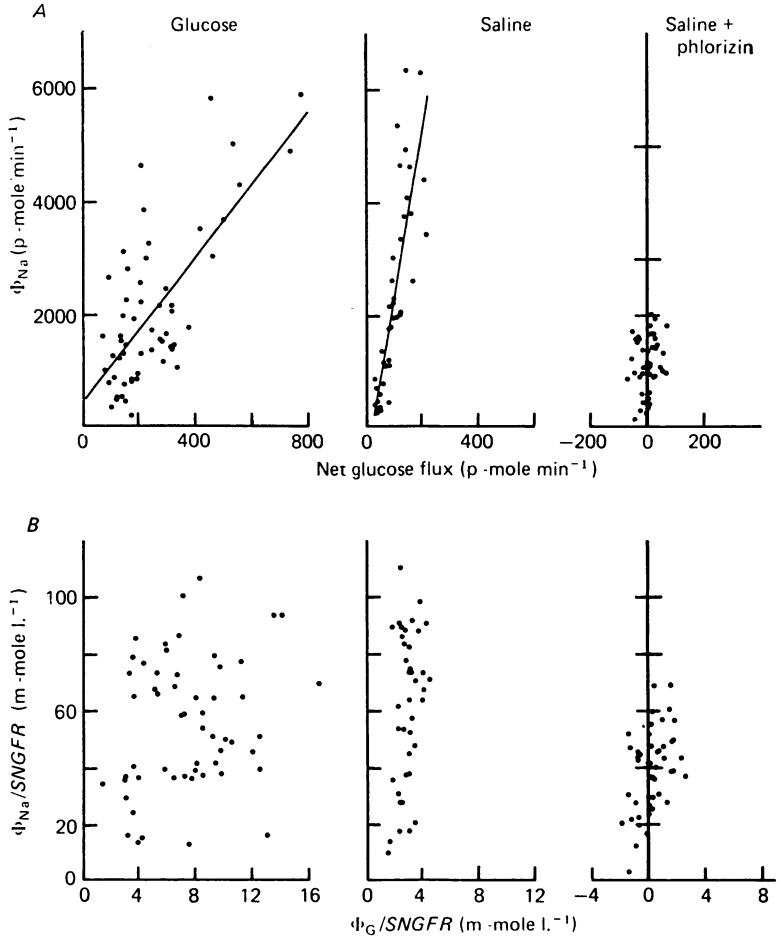

Fig. 8. Relations between *A*, net reabsorptive fluxes of sodium (Φ_{Na}) and glucose (Φ_G); *B*, net reabsorptive fluxes factored by single nephron glomerular filtration rate for sodium ($\Phi_{Na}/SNGFR$) and glucose ($\Phi_G/SNGFR$). The equations of the lines of best fit are

A (*i*) glucose, $y = 413 + 6{\cdot}59x$, $r = 0{\cdot}71$, $P < 0{\cdot}001$;
(*ii*) saline, $y = -688 + 29{\cdot}45x$, $r = 0{\cdot}82$, $P < 0{\cdot}001$;
(*iii*) saline + phlorizin, no significant correlation, $r = 0{\cdot}22$.

The intercept on the ordinate was significantly different from 0 for the glucose group ($P < 0{\cdot}05$) and for the saline group ($P < 0{\cdot}02$); the slopes of the regression lines were significantly different ($P < 0{\cdot}001$).

B (*i*) glucose, no significant correlation, $r = 0{\cdot}19$;
(*ii*) saline, no significant correlation, $r = 0{\cdot}25$;
(*iii*) saline + phlorizin, no significant correlation, $r = 0{\cdot}21$.

The mechanisms by which phlorizin produced the effect on sodium reabsorption are uncertain. The possibility that this is entirely due to a direct effect on coupled glucose/sodium transport seems unlikely since (*a*) quantitatively, the reduction in Φ_{Na} was much greater than that in Φ_G (Fig. 5), (*b*) changes in glucose transport with glucose loading were not associated with corresponding changes in sodium transport (see below), and (*c*) with *in situ* perfusions of pars convoluta with glucose-free solutions, phlorizin caused a reduction in water reabsorption very similar to that induced with glucose perfusions (Bishop *et al.* 1978*b*). Since the relative reductions in sodium and water reabsorption were so similar (Figs. 1, 3 and 5) it seems highly probable that the effects of phlorizin on water were secondary to those on sodium.

Other possible mechanisms involved in the effect of phlorizin on sodium and water were discussed previously (Bishop *et al.* 1978*b*).

Effects of glucose on sodium reabsorption

Although the evidence for stochiometric linkage of proximal tubular glucose reabsorption with sodium is convincing (see references and discussions in Bishop *et al.* 1978*a*, *b*), we have argued that, quantitatively, the fraction of total sodium reabsorption influenced directly by glucose transport is small (Bishop *et al.* 1978*b*). Furthermore, we suggested (Bishop *et al.* 1978*a*) that the quantitative relations between sodium and glucose transport observed in whole kidney preparations arose, primarily, as an indirect consequence of more direct correlation of each with the glomerular filtration rate (i.e. from glomerulo-tubular balance properties which applied to glucose and to sodium, independently).

The present data provide the opportunity to examine glucose/sodium relations at the single nephron level. Fig. 8*A* shows that a high correlation exists between net sodium and glucose fluxes in the saline and glucose-infused groups. However, because the range of net glucose flux was much wider in the glucose-infused group, the regression lines have very different slopes ($P < 0{\cdot}001$), that for the saline group being approximately four times steeper. The absence of significant correlation between net sodium and net glucose fluxes for the saline-phlorizin group is to be expected in view of the abolition of net glucose flux. For the saline-infused rats, it can be calculated that for the whole pars convoluta, some twenty-four to thirty sodium ions were reabsorbed per glucose molecule, in contrast with approximately 6:1 for the glucose-infused rats.

Furthermore, for both the glucose-loaded and saline-infused groups, the ratio Φ_G/Φ_{Na} changes along the length of the tubule, the value at any point being higher in the glucose group than in the saline-infused animals (Fig. 9). Finally, when both Φ_G and Φ_{Na} are divided by *SNGFR* (Fig. 8*B*) in order to allow for any relationship of each with *SNGFR* (i.e. 'glomerulo-tubular balance'), any correlation becomes non-significant.

Thus these data from free-flow micropuncture experiments confirm our previous suggestions derived from whole-kidney (Bishop *et al.* 1978*a*) and *in situ* microperfusion (Bishop *et al.* 1978*b*) studies that total sodium reabsorption is little influenced by glucose transport.

Heterogeneity of pars convoluta

Evidence exists for significant heterogeneity as between superficial and juxtamedullary proximal tubules (Kokko, 1977), and, for superficial nephrons, as between pars convoluta and pars recta with respect to transport of glucose (Tune & Burg, 1971), para-amino hippurate (Tune, Burg & Patlak, 1969), and for chloride permeability relative to sodium (Kokko, 1977). The present study provides some information concerning the distribution of individual transport functions along the length of the superficial pars convoluta.

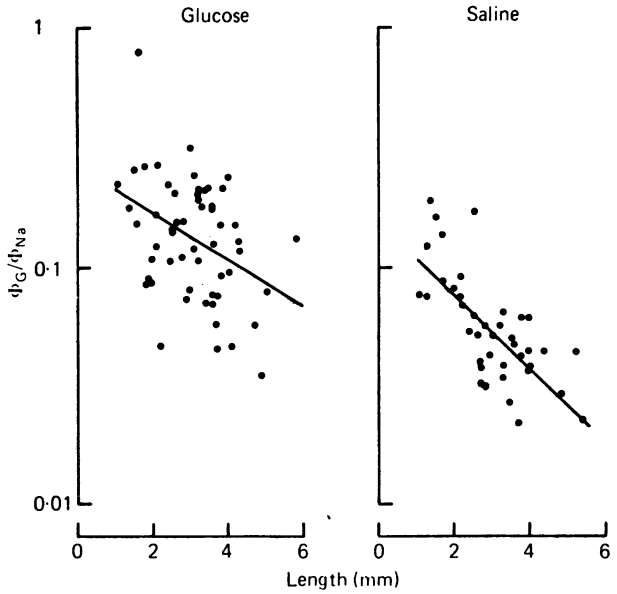

Fig. 9. Relations between distance from glomerulus to puncture site and glucose flux/sodium flux (Φ_G/Φ_{Na}). Data for the saline-phlorizin group are not appropriate since Φ_G was essentially zero (see Fig. 5). The equations of the lines of best fit are

(*i*) glucose, $\log y = 2{\cdot}40 - 0{\cdot}093x$, $r = -0{\cdot}37$, $P < 0{\cdot}01$;
(*ii*) saline, $\log y = 2{\cdot}18 - 0{\cdot}151x$, $r = -0{\cdot}71$, $P < 0{\cdot}001$.

For both glucose and saline groups the intercept on the ordinate was significantly different from 1·0 ($P < 0{\cdot}001$).

In the glucose and saline-infused groups, quantitative reabsorption of sodium (Figs. 3, 5) and water (Figs. 1, 5) appear to be essentially uniform per unit length (unless, as discussed above, the data are interpreted as tentative evidence for lower rates of water reabsorption in the most proximal convolutions). This supports the data of Gyory, Lingard & Young (1974). From the data obtained with *in situ* microperfusions (Bishop *et al.* 1978*b*), it was concluded that there were no significant differences between the glucose-capacity of mid and later convolutions.

In contrast, as discussed above, the glucose-transporting capacity of convolutions close to the glomerulus appears to be significantly greater than that of later proximal convolutions, as may be the case for amino acids (Lingard, Rumrich & Young, 1973). It seems possible that sodium/glucose coupling might be tightest in these most

proximal convolutions. This would be compatible with electrophysiological studies (see Introduction) and with our evidence (Bishop *et al.* 1978*a*, *b*; present paper) that changes in glucose reabsorption influenced only a small proportion of sodium reabsorption in pars convoluta and the whole kidney.

We are indebted to Mr K. Fletcher for technical help and to the M.R.C. (grant no. G973/253/B) and National Kidney Research Fund for financial support.

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