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EFFECTS OF PHLORIZIN ON GLUCOSE, WATER AND SODIUM HANDLING BY THE RAT KIDNEY

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SUMMARY

1. The effect of phlorizin on glucose, water and sodium handling by the kidney in anaesthetized rats was investigated, using clearance techniques, during infusion of saline (200 μl. min^{-1}) or saline to which either low (0·1 μmole kg body weight^{-1} ml.$^{-1}$) or high (1·0 μmole kg body weight^{-1} ml.$^{-1}$) doses of phlorizin had been added.

2. Phlorizin increased the absolute and fractional excretion of glucose, urine osmolality and negative free water clearance; and reduced urine flow rate, glomerular filtration rate (GFR), absolute and fractional excretion of sodium, absolute excretion of sodium, absolute excretion of potassium and absolute and fractional rates of glucose reabsorption.

3. The data indicate that phlorizin has sites of action and effects additional to those on glucose transport in the proximal tubule.

4. Within each series there was a positive correlation between sodium and glucose reabsorption; but the rate of glucose reabsorption was different between each series even though the sodium reabsorption was not.

5. It is suggested that since both sodium and glucose reabsorption correlate with GFR, they may be related via GFR.

6. The data indicate that for the whole kidney any effect of glucose on sodium transport is small relative to total renal handling of sodium.

INTRODUCTION

The conventional description of glucose handling by the mammalian kidney (Smith, 1951), active proximal tubular reabsorption up to a constant maximum transport rate (Tm_G) which, at normal plasma glucose concentrations, sufficiently exceeds filtered load as to permit excretion of glucose-free urine, has required modification in several respects: first, there is evidence that Tm_G is not constant, but alters with changes in glomerular filtration rate (GFR) and in extracellular fluid volume (see reviews by Kurtzman & Pillay, 1973; Morel & de Rouffignac, 1973; and secondly, there is evidence that, as originally proposed by Crane (1962) in respect of glucose transport in the intestine, sugar reabsorption in the kidney may not be a primary, independent process but occurs by a transport mechanism coupled to transmembrane flux of sodium ions.

The abundant evidence that sodium is required for renal absorption of glucose has been presented in several recent reviews (e.g. Ullrich, 1976; Silverman, 1976). But, the sparse evidence as to whether the converse applies, that glucose is necessary for

normal tubular reabsorption of sodium, is less consistent. Thus, while demonstrations of an effect of glucose on proximal transtubular potential difference (Kokko, 1973; Frömter & Gessner, 1974; Cardinal, Lutz, Burg & Orloff, 1975) have been interpreted in terms of an influence of glucose on sodium co-transport, such studies provide no information as to the quantitative significance of glucose-influenced sodium movement relative to total proximal tubular sodium reabsorption. Such information as is available from micropuncture experiments on rat kidney is inconsistent, and includes descriptions of a marked effect of glucose on proximal tubular reabsorption of fluid (Weinman, Suki & Eknoyan, 1976) and no significant effect (Green & Giebisch, 1975; Bishop, Green & Thomas, 1975, 1978).

Similarly, at the level of the whole kidney, few studies have been designed specifically to examine the influence of primary changes in glucose handling on sodium excretion; and again, the results vary in respect of quantitative significance and of interpretation. Thus, in experiments on the isolated, perfused rat kidney, changes in glucose transport have been shown to be accompanied by changes in sodium handling, but the effect has been ascribed to indirect, metabolic consequences of changing glucose availability as substrate rather than to sodium-glucose co-transport (Trimble & Bowman, 1973; Ross, Frega & Leaf, 1976).

The present study presents data concerning glucose and fluid handling at the level of the whole kidney of the rat as influenced by phlorizin, considered to be a relatively specific competitive inhibitor (Frasch, Frohnert, Bode, Baumann & Kinne, 1970) of sodium-dependent glucose transport in the kidney (Lotspeich, 1960; Alverado & Crane, 1964). The protocol has been influenced by three main considerations: (*a*) to vary renal glucose reabsorption (by use of phlorizin) while minimizing the effects on glucose handling induced by changes in body fluid volume (see above); (*b*) to examine possible effects of phlorizin on renal functional parameters (GFR, water and solute handling) additional to primary changes in glucose; and (*c*) to provide information from whole kidney experiments pertinent to planning micropuncture experiments on nephron segments. Preliminary data concerning micropuncture experiments on the proximal tubule (Bishop *et al.* 1975) and loop of Henle (Bishop, Green & Thomas, 1976) have been reported.

METHODS

Experiments were performed on male Sprague–Dawley rats weighing 140–180 g, which had been fasted for 16 h before the experiment but had been allowed free access to water. The rats were anaesthetized by an intraperitoneal injection of Inactin (Promonta Corp., Hamburg, Germany), 120 mg kg body weight^{-1}, and placed on a temperature-controlled operating table which maintained body temperature at 37 °C.

Catheters (Portex plastics, Hythe, Kent) were inserted into the left jugular vein (PP10) for administration of priming and maintenance infusions and into the right carotid artery (PP50) for recording blood pressure (via a Statham P23 DC transducer and a Grass P7 polygraph). To ensure a clear airway, a tracheostomy was performed. The bladder was catheterized via a suprapubic incision taking care to ensure that the bladder dead space was reduced to a minimum and that the urethra was tied.

Saline solutions (150 mM) were administered I.V. as a priming dose (0·8 ml. containing [^{3}H]inulin, 20 μc ml.$^{-1}$) during the surgical procedures; and as sustaining infusion at 200 μl. min^{-1} (containing [^{3}H]inulin, 5 μc ml.$^{-1}$) beginning as soon as the jugular catheter had been inserted. Three series of experiments were performed.

(1) *Controls.* Priming dose of saline and maintenance infusion as above.

(2) *Phlorizin, low dose.* Priming dose of saline contained phlorizin (K & K Rare Chemicals, Liverpool) 2 μmole kg body weight^{-1}; maintenance infusion contained phlorizin, 0·1 μmole kg body weight^{-1} ml.$^{-1}$.

(3) *Phlorizin, high dose.* Priming dose of saline contained phlorizin, 20 μmole kg body weight^{-1}; maintenance infusion contained phlorizin 1·0 μmole kg body weight^{-1} ml.$^{-1}$.

The first hour of continuous infusion after completion of surgery was allowed for equilibration. Subsequently, urine was collected every 15 min for the next 1·75 hr into preweighed glass tubes containing a little liquid paraffin. Urine volume was determined gravimetrically. Blood samples (obtained from the tail vein during, and by direct cardiac puncture at the end of, the experiment) were heparinized and centrifuged to give plasma samples for analysis.

[^{3}H]inulin was counted in a liquid scintillation counter (Intertechnique model SL30, Paris) with a scintillant containing 5 ml. PCS (Amersham Searle, Arlington Heights, Illinois, U.S.A.), 5 ml. toluene A.R. and 0·5 ml. deionized water. Ten μl. aliquots of urine and plasma were counted. Sodium and potassium concentrations were measured in suitably diluted samples of blood and urine by flame photometry (EEL 150; Halstead, Surrey) and osmolality measured by freezing point depression (Advanced Osmometer Model 2LS, Advanced Instruments, Mass., U.S.A.).

Glucose was measured using the hexokinase, glucose-6-phosphate dehydrogenase method (Bergermeyer, 1963): 40 ml. ATP (8·25 μmole ml.$^{-1}$); 60 ml. NADP (2·62 μmole ml.$^{-1}$); 25 ml. $MgCl_2$ (8·2 μmole ml.$^{-1}$ in Tris buffer pH 7·6); 5 ml. hexokinase (88 units ml.$^{-1}$] and 170 ml. Tris buffer (0·1 M, pH 7·6) were mixed. To 3 ml of this mixture were added 25 μl. of a suitably diluted sample of plasma or urine and 25 μl. glucose-6-phosphate dehydrogenase (70 units ml.$^{-1}$). After 30 min at room temperature the fluorescence was read against appropriate standards on a spectrophoto-fluorimeter (Aminco, Silver Springs, Md. U.S.A.) using excitation at 360 nm and emission 440 nm.

GFR was calculated as clearance of inulin, $C_{in} = (U_{in}\ V)/P_{in}$, and then used to calculate the filtered load of a substance = $C_{in}.P_x$; reabsorptive rate = $(C_{in}.P_x)-(UV_x)$; fractional reabsorption = $((C_{in}.P_x)(U_xV))/(C_{in}.P_x)$; and fractional excretion = 1 − fractional reabsorption where U_{in} is the urinary and P_{in} the plasma, concentration of inulin; U_x the urinary, and P_x the plasma, concentration of substance X; and V the urinary flow rate. Urine and plasma osmolalities were used to calculate negative free water clearance as

$$-C_{H_2O} = -V\left[1-\frac{U_{osm}}{P_{osm}}\right].$$

[C_{H_2O} is the free water clearance, P_{osm} is the plasma, and U_{osm} the urinary, osmolality].

Values are presented as mean ± S.E. Statistical analyses are described in Results.

RESULTS

All rats maintained a mean blood pressure greater than 100 mmHg (14 kPa); there were no significant differences between the series. Plasma sodium and potassium concentrations were not significantly influenced by phlorizin (Table 1). Plasma glucose concentration was significantly lower in both phlorizin series (Table 1), presumably reflecting urinary loss of glucose.

Mean values for GFR, urine flow rate and for renal parameters concerning sodium and glucose handling are presented for all collection periods in Figs 1–3. Analysis of variance was performed in order to examine the effects of dose of phlorizin and of duration of infusion on the various renal parameters. With few exceptions. duration of infusion of phlorizin proved to have no consistent, statistically significant effects on these parameters; i.e. the initial equilibration period was adequate. Accordingly, subsequent mean values in the text are derived by pooling the values from each collection period.

In contrast, dose of phlorizin had a significant influence on the magnitude of the response for most parameters (Table 2). Subsequent textual statements concerning statistical significance relate to Table 2.

Flow rate was highest (mean 168 μl. min^{-1}) in the control series; with phlorizin, flow significantly decreased to mean values of 142 in series 2 and 121 μl. min^{-1} in series 3. GFR was also significantly lowered by increasing dose of phlorizin from a mean 1·68 ml. min^{-1} with controls to 1·63 in series 2 and to 1·48 ml. min^{-1} in series 3. Urine osmolality increased from a mean of 319 in the control series to 372 in series 2 and 396 in series 3 (all m-osmole kg water^{-1}); but there were no significant differences in plasma osmolality between series (Table 1). The negative free water clearance was significantly increased from a mean of 6 for the control series to 25 μl. min^{-1} for series 2, but there was no further change in series 3 (mean 22 μl. min^{-1}).

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

Series	n	Sodium (m-mole l.$^{-1}$)	Potassium (m-mole l.$^{-1}$)	Glucose (m-mole l.$^{-1}$)	Osmolality (m-osmole kg water^{-1})
1. Control	6	133·5 ± 1·5	5·77 ± 0·21	4·52 ± 0·45	305 ± 6
2. Phlorizin (low dose)	7	131·0 ± 2·3	6·10 ± 0·78	3·58 ± 0·18*	304 ± 2
3. Phlorizin (high dose)	7	135·3 ± 2·4	5·68 ± 0·54	3·36 ± 0·15*	302 ± 3

The high potassium values (mean ± S.E.) in series 2 are due to one high value which may have resulted from haemolysis of the sample.

* Significantly different ($P < 0·05$) from control values (t test)

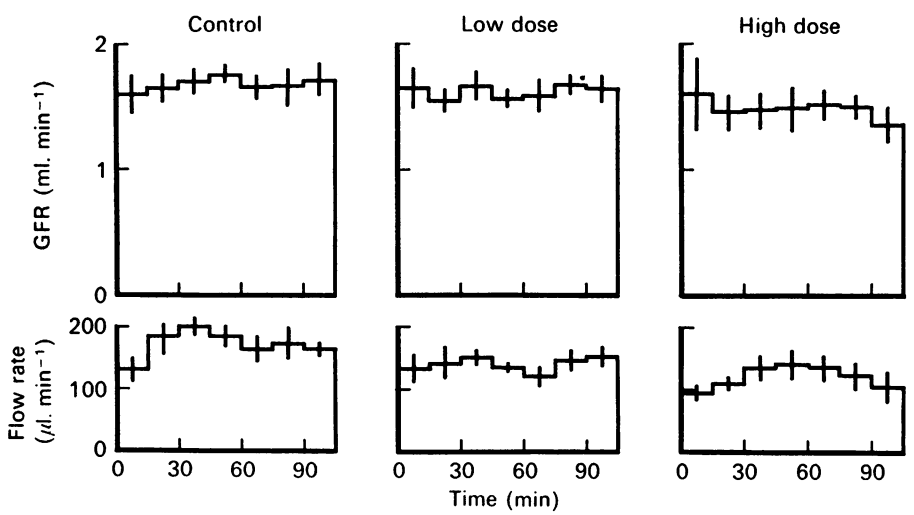

Fig. 1. Effects of low and high doses of phlorizin (see Methods) on glomerular filtration rate (GFR) and urinary flow rate, compared with control experiments. Time zero = end of 1 hr equilibration period. Values represent mean ± S.E. Numbers of experiments are given in Table 1.

Sodium (Fig. 2)

Although plasma sodium concentrations were not significantly different, the lower GFR in the two phlorizin series caused reductions in the filtered load of sodium and were accompanied by significant decreases in the rate of sodium excretion, the

extent of the latter being significantly greater (Table 2) with the higher dose of phlorizin. In contrast, the absolute rate of sodium reabsorption (difference between filtered load and sodium excretion) was not significantly altered.

Expressed as a fraction of the filtered load, sodium excretion decreased with increasing dose of phlorizin, i.e. fractional sodium reabsorption increased (Table 2, Fig. 2).

TABLE 2. Effect of increasing doses of phlorizin on renal parameters (analysis of variance)

Parameter	Degrees of freedom	Variance	F	P	Effect
GFR (ml. min^{-1})	2	0·499	4·54	< 0·05	Decreased
Urine flow (μl. min^{-1})	2	29766	13·72	< 0·001	Decreased
Urine osmolality (m-osmole kg $water^{-1}$)	2	65907	5·61	< 0·01	Increased
$-C_{H_2O}$ (μl. min^{-1})	2	4290	4·43	< 0·05	Increased
Sodium excreted (μmole min^{-1})	2	365·9	10·43	< 0·001	Decreased
Sodium reabsorbed (μmole min^{-1})	2	3936·6	1·80	NS	—
Fractional sodium reabsorption	2	40·17	4·43	< 0·05	Decreased
Potassium excreted (μmole min^{-1})	2	4·16	6·84	< 0·01	Decreased
Potassium reabsorbed (μmole min^{-1})	2	10·60	2·10	NS	—
Fractional potassium reabsorption	2	156·0	2·68	NS	—
Urinary glucose concentration (m-mole $l.^{-1}$)		106·39	146·9	$\ll$ 0·001	Increased
Glucose excreted (μmole min^{-1})	2	116·0	305·0	$\ll$ 0·001	Increased
Glucose reabsorbed (μmole min^{-1})	2	386·2	106·5	$\ll$ 0·001	Decreased
Fractional glucose reabsorption	2	55904	178·3	$\ll$ 0·001	Decreased
Glucose reabsorption/GFR (μmole $ml.^{-1}$)	2	138·3	174·8	$\ll$ 0·001	Decreased

Potassium

The filtered load and rate of excretion of potassium decreased with increasing dose of phlorizin but to values such that potassium reabsorption, both absolute and fractional, did not change significantly (Table 2). The quantitative data are not presented.

Glucose (Fig. 3)

As expected, phlorizin had significant effects on glucose handling by the kidney. Glucose concentration in the urine increased from a mean of 0·19 in controls to 10·77 in series 2 and 30·43 (all m-mole $l.^{-1}$) in series 3. The absolute and fractional excretion of glucose increased with increasing dose of phlorizin and absolute

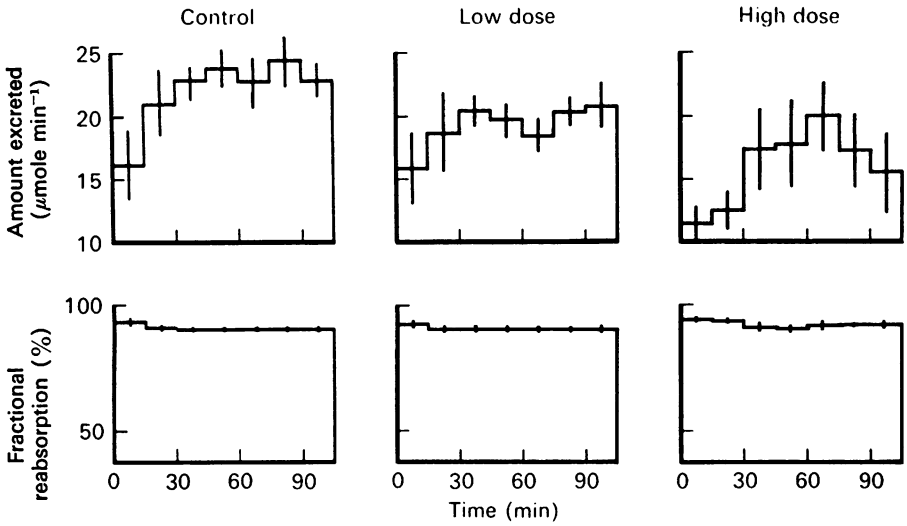

Fig. 2. Effects of low and high doses of phlorizin on urinary excretion and fractional reabsorption (as %) of sodium, compared with control experiments. Details as in Fig. 1.

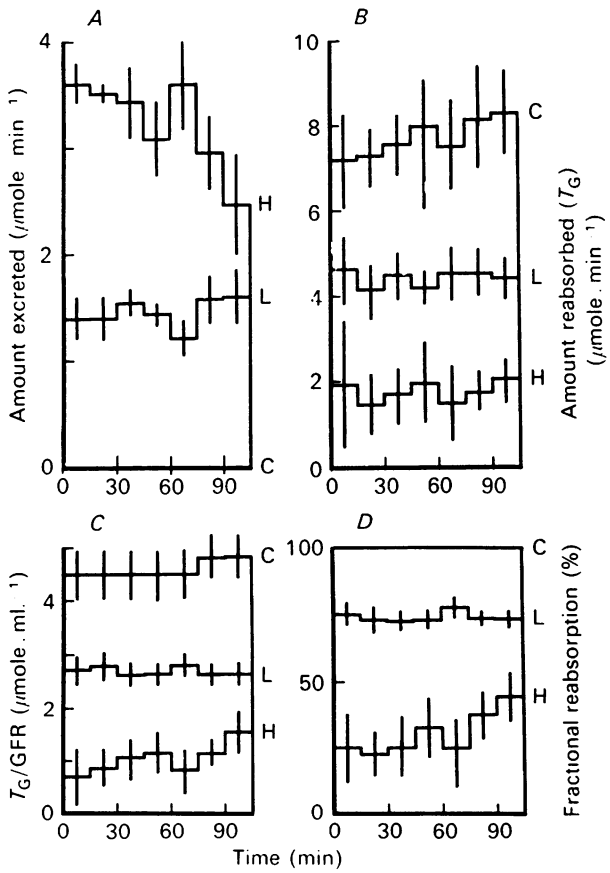

Fig. 3. Effects of low and high doses of phlorizin on renal glucose handling, compared with controls: *A*, glucose excretion rate; *B*, glucose reabsorption rate (T_G); *C*, glucose reabsorption/glomerular filtration rate (T_G/GFR); *D*, fractional reabsorption (as %). Details in Fig. 1.

reabsorption of glucose concomitantly decreased. Fractional reabsorption of glucose averaged 99·6% in the control series; this was significantly reduced to about 75% in series 2 and to about 30% in series 3. For all of these renal aspects of glucose handling the statistical significance was very high ($P \ll 0{\cdot}001$). To allow for effects of changes in GFR on glucose absorption, T_G/GFR (the amount of glucose reabsorbed factored by GFR) is also plotted (Fig. 3); the differences in glucose reabsorption between the series remained very highly significant.

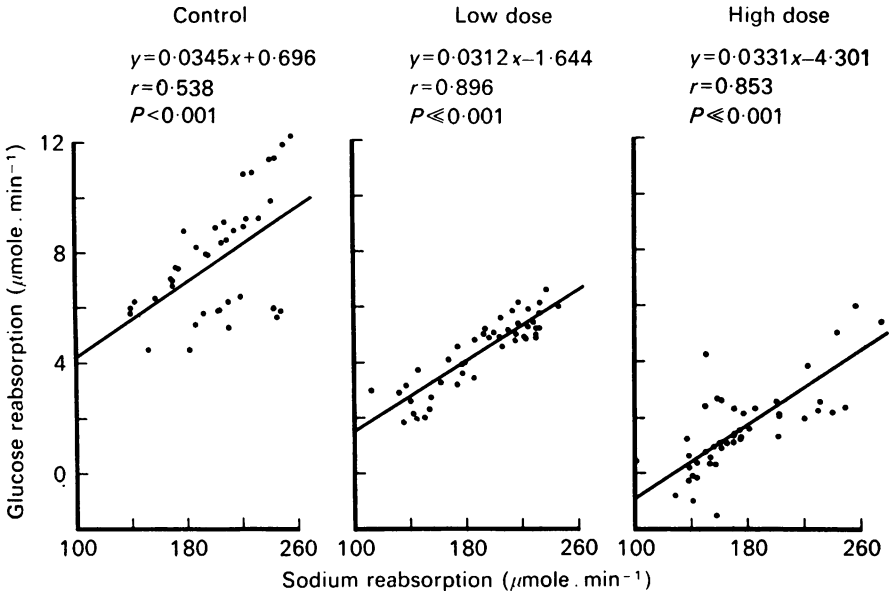

Fig. 4. Relation between glucose reabsorption rate and sodium reabsorption rate in controls and in experiments with low and high doses of phlorizin. Each value represents an individual urine collection period. Numbers of experiments as given in Table 1.

When the relationship between glucose reabsorption and sodium reabsorption was examined, it was evident that for each series there was a highly significant positive correlation (Fig. 4). The slopes of the regression lines were not significantly different between series whereas the intercept was ($P < 0{\cdot}001$ for each comparison). Thus even when phlorizin is present, there is a significant relation between glucose and sodium reabsorption.

DISCUSSION

Although the conventional classical description of glucose handling by the kidney includes complete reabsorption by the proximal tubules at filtered loads below Tm_G, the use of sufficiently sensitive glucose assay methods in the present and other investigations (Rhode & Deetjen, 1968; Fröhnert, Höhmann, Zwiebel & Baumann, 1970; von Baeyer, von Conta, Haberle & Deetjen, 1973; von Baeyer, 1975) makes it clear that even at normal plasma glucose concentrations, some glucose is always present in the urine of rats, albeit at low (approximately 0·2 m-mole $l.^{-1}$) concentrations. The significance of this in relation to glucose handling by proximal

convoluted tubule (Bishop *et al.* 1978) and by more distal segments of the nephron (J. H. V. Bishop, R. Green & S. Thomas, to be published) will be discussed elsewhere.

Effect of phlorizin on renal function

Increasing the dose of phlorizin increased the absolute and fractional excretion of glucose, urinary osmolality, and negative free water clearance; and reduced GFR, urine flow rate, absolute and fractional excretion of sodium, absolute excretion of potassium and absolute and fractional rates of glucose reabsorption.

The effect of phlorizin on glucose excretion has been known for many years (see Smith, 1951; Lotspeich & Woronkow, 1958; Lotspeich, 1960) although the dose used in different species has varied widely; to our knowledge, no dose–response data have been published for the rat. Huang & Woosley (1968) presented data for the rat using only our lower doses, which in dogs gives inhibition of glucose reabsorption by 70–80 % (Lotspeich & Woronkow, 1958). In dogs, a tenfold increase in phlorizin dose had little further effect on reabsorption. Smith (1951) collected data which showed that if given in sufficiently high dose intravenously (349 μmole as a single injection), complete inhibition of glucose reabsorption occurred in both dog and man but this was accompanied by 'a marked reduction in all clearances by profound circulatory effects'.

As compared with previous data on dogs the present results show that, for comparable low doses, phlorizin has a less marked effect on glucose reabsorption in rats than in dogs. However, because the range of inhibition of glucose reabsorption proved to be sufficient for the intended examination of the relationship between glucose and sodium handling, as discussed below, and because increasing dosage of phlorizin was accompanied by other effects on the kidney (Table 2) we have preferred not to use larger doses of phlorizin.

The effect of phlorizin on urine flow rate was surprising. Glycosuria might be expected to produce an increased urine flow as an osmotic diuretic effect, even though the amount of glucose excreted was small relative to the glycosuria which occurs at elevated plasma glucose levels. The reasons for this unexpected decrease in flow rate are not known but it seems most likely to be secondary to the decrease in GFR.

Although decrease of GFR with phlorizin has been noted at massive doses (see Smith, 1951), there seems to be no previous report concerning lower doses such as those used here. Close inspection of the data from individual experiments reported by others (Lotspeich & Woronkow, 1958; Lotspeich, 1960) shows, however, that the effect may have been present but was not discussed. The possibility that the decrease in GFR is secondary to a negative water balance in the initial equilibration period (1 hr), due to a glucose osmotic diuresis, may be discounted; during this period, urine flow remained so low that any such effect on water balance was trivial. Alternative speculations are that phlorizin might exert direct or indirect (via changes in luminal fluid composition) effects on the macula densa with consequent feed-back reductions in GFR (Thurau & Levine, 1971); or that circulatory consequences of phlorizin administration (Smith, 1951) might contribute to changes in cardiac output and so to changes in GFR. In this respect it has been shown that plasma glucose levels above 180 m-mole $l.^{-1}$ are accompanied by increased GFR (Brøchner-Mortensen, 1973); whether the converse applies is not known.

As urine osmolality rose with phlorizin dose, the negative free water clearance also increased. It has been proposed that such changes in negative free water clearance indicate changes in the ascending limb of the loop of Henle or the distal tubule (Goldberg, 1973). While such an interpretation is controversial, it does seem reasonable to infer that phlorizin may be acting at sites other than the proximal tubule and on substances other than glucose. But whether this effect represents a direct action of phlorizin on nephron permeability characteristics or secondary effects of glucose on distal tubular fluid, or effects of phlorizin on other body parameters cannot be determined. The results of our separate study on proximal tubular handling of glucose, ions and water (Bishop *et al.* 1978) provide more direct evidence that even within this tubular segment, phlorizin has effects additional to those on glucose transport.

Effect of glucose on sodium

The absolute amounts of sodium and potassium reabsorbed were not different between the three series (Table 2); although because the lowering of GFR in the phlorizin experiments led to reductions in filtered load, fractional reabsorption of sodium increased with increasing doses of phlorizin. Fractional reabsorption of potassium was not significantly different between the series. Thus the thesis that reduced glucose reabsorption substantially alters sodium reabsorption cannot be sustained for the whole kindey, at least under the circumstances of the present study. The absolute amount of sodium reabsorbed was not significantly different between the three series even though the amount of glucose reabsorbed fell by up to 70–80 %. It must be stressed, however, that in absolute quantity, the variation in the amount of glucose reabsorbed was only 6 μmole min^{-1}; and that if any effect of glucose is mediated solely via the proposed 1:1 stoichiometric relationship between glucose and sodium (Ullrich, Rumrich & Klöss, 1974), the consequent change in the amount of sodium reabsorbed would have been difficult to detect in a total of approximately 190 μmole min^{-1}. The only valid conclusion is that any effect of glucose on sodium must be small relative to the total amount of sodium reabsorbed. This is in agreement with the evidence from experiments on frog kidney (Vogel & Kröger, 1966; Vogel, Tervooren & Stoeckert, 1966) in which, while glucose transport was influenced by primary changes in sodium transport, sodium was not significantly affected by primary changes in glucose; and also with the conclusions of our micropuncture study on proximal tubule (Bishop *et al.* 1978).

However, while the values for sodium reabsorption (means and range of variation) were essentially similar as between the three series of experiments, there was a sufficiently wide range of values within any one series to permit examination of a possible relationship between glucose and sodium reabsorption for that series (Fig. 4). This showed an apparent relationship between sodium and glucose reabsorption as was described by Kurtzman, White, Rogers & Flynn (1972). This might be interpreted as evidence supporting a direct causal relationship. But, in our view, the present data are more reasonably interpreted on the basis that the relation between glucose and sodium is, at least in part, an indirect consequence of a more direct relationship of each to another parameter, namely GFR. Figs. 5 and 6 show that both sodium and glucose reabsorption correlate well with GFR as previously described (Kurtzman *et al.* 1972; Kurtzman & Pillay, 1973). It should be noted that

the relationship between sodium reabsorption and GFR (Fig. 5) is not significantly different between series either in slope or intercept and that the correlation was very high ($P \geqslant 0{\cdot}94$ in all series), indicating that in all three states of glucose reabsorption, the kidney was achieving glomerulo-tubular balance for sodium. Fig. 6 shows that there was also a positive correlation between glucose reabsorption and GFR

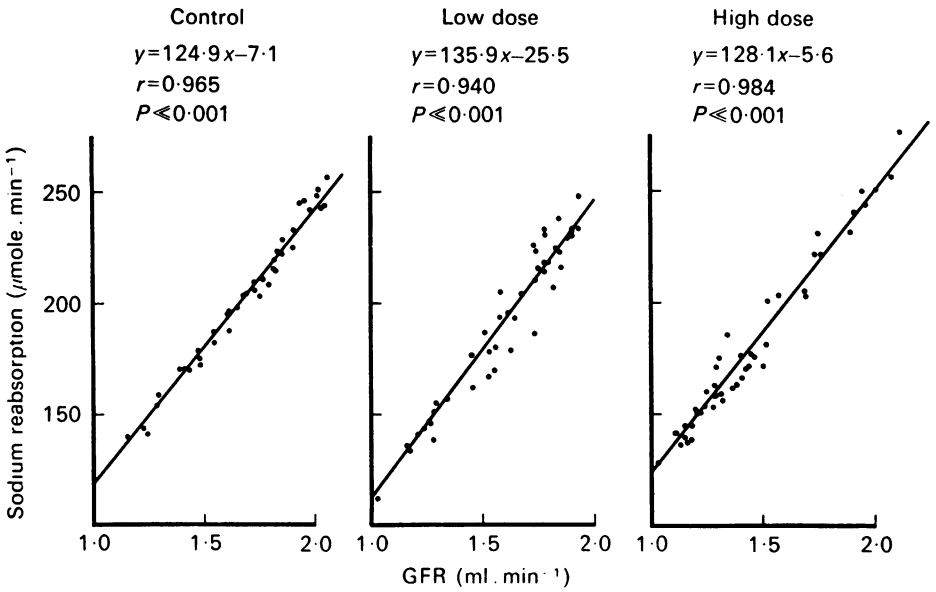

Fig. 5. Relation between sodium reabsorption rate and glomerular filtration rate (GFR) in controls and in experiments with low and high doses of phlorizin. Details as in Fig. 4.

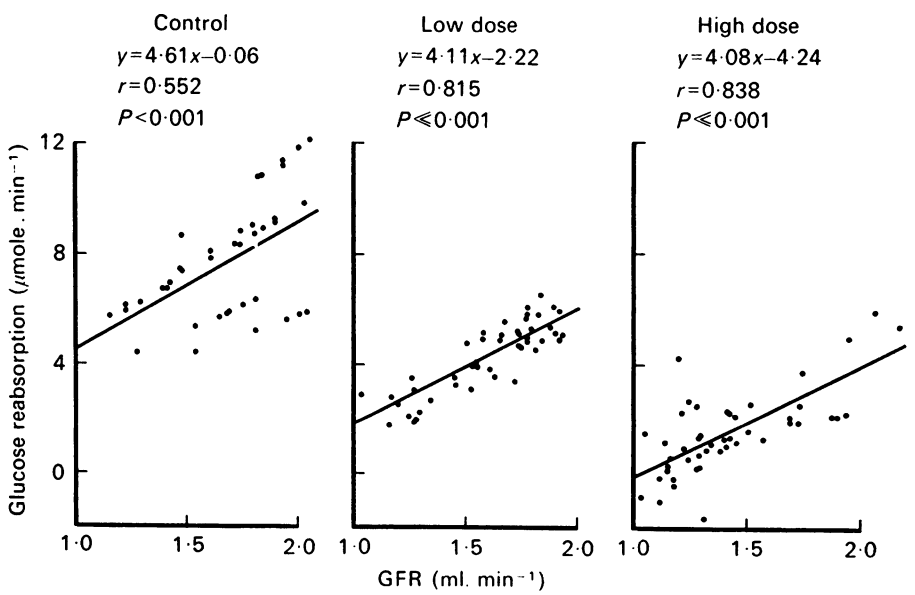

Fig. 6. Relation between glucose reabsorption rate and glomerular filtration rate (GFR) in controls and in experiments with low and high doses of phlorizin. Details as in Fig. 4.

($P < 0{\cdot}001$ in all series) and that again there were no significant differences between the slopes of the regression lines. However, in contrast to the sodium/GFR data, intercepts were significantly different ($P < 0{\cdot}001$ for each comparison), a finding similar to the relationship between glucose and sodium reabsorption (cf. Fig. 4).

Following the classical studies of renal glucose handling (Shannon & Fisher, 1938), it has been conventional to factor glucose reabsorption by GFR in order to allow for differences in GFR between animals. More recently, numerous workers (van Liew, Deetjen & Boylan, 1967; Keyes & Swanson, 1971; Kurtzman *et al.* 1972; Schultze & Berger, 1973; Kwong & Bennett, 1974) have shown that there is no clear Tm_G when

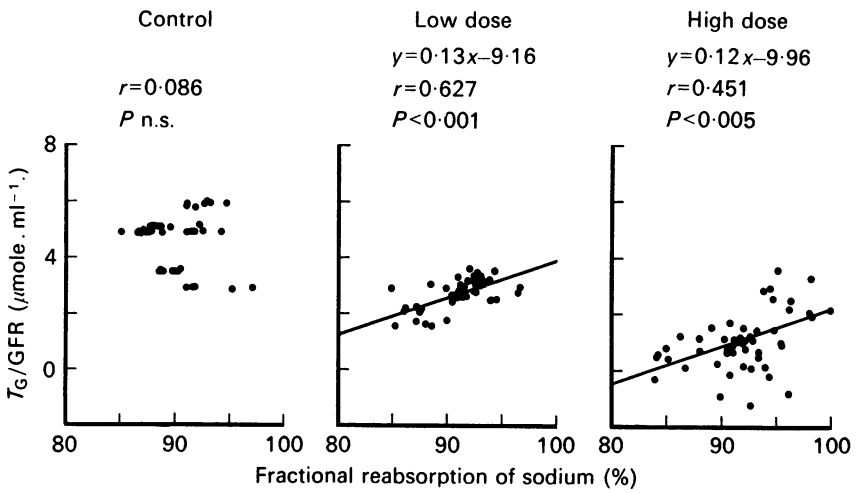

Fig. 7. Relation between glucose reabsorption rate/glomerular filtration rate (T_G/GFR) and fractional reabsorption of sodium (as %) in controls and in experiments with low and high doses of phlorizin. Details as in Fig. 4.

changes in GFR occur and so have used the same factoring. Using this procedure in examining glucose and sodium handling, an inverse relationship has been demonstrated between glucose reabsorption/GFR and fractional excretion of sodium (Kurtzman *et al.* 1972; Lennon, Lemann, Piering & Larson, 1974). Kurtzman *et al.* (1972) showed this for circumstances where the filtered load of glucose was greater than 1·5× the reabsorbed load, whereas Lennon *et al.* (1974) demonstrated the relationship in man in conditions where the individuals were 'non-glycosuric' to conventional clinical tests. Although, as discussed above, we consider that our data indicate that absolute glucose and sodium reabsorption are both related to GFR, we also present the glucose data in the form of glucose reabsorption/GFR to permit comparison with the results of previous workers (Fig. 7). In the control series the correlation between glucose reabsorbed/GFR and fractional sodium reabsorption was low ($r = 0{\cdot}086$) and not significant; indeed when individual experiments are examined, it is apparent that fractional reabsorption of sodium may vary widely even when glucose reabsorption/GFR is relatively constant. When phlorizin was infused and glucose present in increased amounts in the urine, there was a significant correlation between glucose reabsorption/GFR and fractional reabsorption of sodium (Fig. 7). There were no significant differences between the two series either in the

slope of the regression line or in its intercept. Kurtzman *et al.* (1972) and Lennon *et al.* (1974) interpreted their data as showing interdependence of sodium and glucose reabsorption. But if, as discussed above, absolute glucose and sodium reabsorption are related mainly via GFR in the circumstances of our experiments, then the relation between glucose reabsorption/GFR and fractional reabsorption of sodium within each phlorizin series may represent an indirect consequence of some other effect; for example, it is conceivable that the osmotic effects of increasing glucose concentration in distal parts of the nephron may secondarily alter the amount of sodium excreted.

In apparent contrast with our conclusions, there appears to be no doubt from electrical studies on proximal tubule (Kokko, 1973; Frömter & Gesssner, 1974; Burg, Patlak, Green & Villey, 1976) and from studies on isolated brush borders (Kinne, 1975; Kinne, Murer, Kinne-Saffran, Thees & Sachs, 1975) that there is some influence of glucose on sodium; but such studies provide no information as to the quantitative significance of this effect relative to the total renal reabsorption of sodium. In studies on proximal tubular handling of fluid and glucose, it has been argued that the data provide evidence of a direct relationship between sodium and glucose (Burg *et al.* 1976; Weinmann *et al.* 1976), but the quantitative significance of this has been disputed (Green & Giebisch, 1975). Proximal tubular handling of sodium and glucose is examined in detail in another paper (Bishop *et al.* 1978).

In conclusion, we consider that for the whole kidney, our data indicate that any effect of glucose of sodium reabsorption is relatively small, and that it may include indirect effects acting through changes in GFR, in osmolality of the distal tubular fluid, or even in renal metabolism. This is in agreement with the work of Vogel & Kroger (1966) and Vogel *et al.* (1966) on frog kidney. Considering that glucose reabsorption is normally essentially complete in the first part of the proximal tubule (Rohde & Deetjen, 1968), that nephron reabsorption of sodium is influenced by multiple factors (Green & Giebisch, 1976) and that the sequential reabsorption of sodium along the nephron displays different characteristics (Jacobson & Kokko, 1976), this is not surprising. Furthermore, even for glucose, segments of the nephron distal to the proximal convoluted tubule possess transport properties (von Baeyer, 1975; Bishop *et al.* 1976) that may influence excretion by the whole kidney. It would appear that while glucose may be required for a fraction of the total sodium reabsorption, this is not a quantitatively significant factor in sodium balance as determined for the whole kidney.

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