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The influence of dietary potassium on the renal tubular effect of hydrochlorothiazide in the rat

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1 The influence of dietary potassium on the natriuretic effect of hydrochlorothiazide was investigated in conscious rats which had access to 0.46 M NaCl solution; the intake of saline was used as an index of the natriuresis.

2 Control rats drank very little saline (<1 mmol $24\,h^{-1}$), whereas animals given hydrochlorothiazide in the food (35 mg kg^{-1} dry weight) increased their saline intake to approximately 10 mmol $24\,h^{-1}$. In a third group of rats, on a high-potassium diet (360 mmol kg^{-1} dry weight vs 60 mmol kg^{-1} dry weight), the same dose of hydrochlorothiazide increased the saline intake to only 2 mmol $24\,h^{-1}$.

3 In order to investigate the renal mechanisms involved in these effects, animals were anaesthetized and prepared for micropuncture. Collections were made from late surface convolutions of proximal tubules and from early and late regions of distal tubules.

4 Total glomerular filtration rate, single-nephron filtration rate, and the delivery of sodium to the end of the proximal tubule and to the beginning of the distal tubule were similar in the three groups of rats.

5 In rats on a normal diet, hydrochlorothiazide treatment was associated with an increased delivery of sodium to the end of the distal tubule. No such increase was seen in thiazide-treated rats on a high potassium intake.

6 It is concluded that a high potassium intake reduces the natriuretic effect of hydrochlorothiazide as a result of interference with thiazide-induced inhibition of sodium reabsorption in the distal tubule. The effect of potassium does not depend on changes in sodium handling in other nephron segments. The possible roles of aldosterone and distal tubular potassium secretion in mediating this effect are discussed.

Introduction

The principal effect of thiazide diuretics is thought to be inhibition of sodium chloride reabsorption in the early part of the distal tubule (Costanzo, 1985; Velazquez & Wright, 1986), resulting in a natriuresis. Unfortunately, a frequent side-effect of treatment with thiazides is a reduced plasma potassium concentration. In order to try to prevent the thiazide-induced hypokalaemia it is common practice to administer potassium supplements during treatment with these diuretics. It is of interest, therefore, that a recent study has indicated that in rats the addition of potassium to the diet can reduce markedly the natriuretic effect of thiazides (Olesen, 1982).

In the present investigation we have confirmed the inhibitory action of potassium on thiazide-induced natriuresis and, in order to shed light on the mechanism of the phenomenon, we have used micropuncture techniques to identify the region of the nephron in which it occurs. Some of the results have been presented in a preliminary communication (Shirley & Walter, 1984).

Methods

Male Long Evans rats, weighing 180–200 g at the start of the study, were kept in metabolism cages (Jencons, Leighton Buzzard) in a room illuminated from 15 h 00 min to 03 h 00 min. Animals were divided into three groups: groups 1 and 2 were maintained on a wet mash diet with a sodium content of 100 mmol kg^{-1} dry weight and potassium content of 60 mmol kg^{-1} dry weight (Special Diet Services, Witham); group 3 was fed the same basic diet but with KCl added so that the final potassium content was 360 mmol kg^{-1} dry weight. All rats had free access to both food and deionised water.

Group 1 animals (controls) remained on the same

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diet throughout the study. After a 4 day equilibration period and a 3 day control period, animals of groups 2 and 3 were given hydrochlorothiazide (Merck, Sharp & Dohme) in their food at a concentration of 35 mg kg^{-1} dry weight, equivalent to a daily dose of approximately 2 mg kg^{-1} body weight.

When diuretics are administered chronically, the resulting sodium deficit initiates compensatory changes in the nephron which mask the full expression of the natriuretic effect (Walter & Shirley, 1986). To prevent this occurring in the present experiments, all rats were allowed access to 0.46 M NaCl solution, in addition to their drinking water. It has been shown that rats will not normally drink significant amounts of saline of this strength, but that they will do so if their sodium balance is threatened; they then drink just sufficient saline to remain in sodium balance (Richter, 1936; Olesen, 1982). Thus in the present experiments the natriuretic effect of hydrochlorothiazide was not compromised by a sodium deficit, and the intake of 0.46 M NaCl solution was used as an index of the natriuretic response.

Measurements of body weight, food intake, urinary electrolyte excretion and saline intake were made daily throughout the period in metabolism cages.

After the equilibration and control periods, animals of all three groups were maintained for a further 7–9 days on their respective regimes and were then used in micropuncture experiments. At 08 h 00 min each rat was anaesthetized with Inactin (Byk Gulden, Konstanz, FRG; 120 mg kg^{-1} body weight, i.p.) and surgically prepared for micropuncture as described previously (Walter *et al.*, 1979; Walter & Shirley, 1986). Sodium chloride solution (150 mmol l^{-1}) was infused intravenously throughout at a rate of 6 ml h^{-1} kg^{-1} body weight, except during the final hour of surgical preparation when the infusion rate was adjusted to provide an extra volume of saline equivalent to 0.5% of the body weight (to replace surgical losses).

Following surgery, [^{3}H]-inulin (Amersham International, Amersham) was added to the infusion (100 μCi primer, 100 μCi per h), and after 1 h of equilibration a 3 h experimental period was initiated. Urine collections were made throughout, plasma samples taken intermittently, and micropuncture collections made from late proximal convoluted tubules and from early and late regions of distal tubules, according to procedures described previously (Walter *et al.*, 1979; Walter & Shirley, 1986).

Analyses

Urine and plasma electrolyte concentrations were measured by flame photometry (1L, Warrington; model 543). Packed cell volumes were determined using microhaematocrit tubes; plasma protein concentration was measured by the method of Lowry *et al.* (1951); and plasma aldosterone concentration was measured by radioimmunoassay (James & Wilson, 1976).

The volumes of tubular fluid collections were measured using calibrated constriction pipettes. Tubular fluid Na and K concentrations were determined by helium glow photometry (Aminco, Silver Spring, Maryland, U.S.A). The activities of [^{3}H]-inulin in tubular fluid, urine and plasma were determined in Aquasol 2 scintillation cocktail (Du Pont, Stevenage) by β emission spectroscopy (Packard Tri-Carb).

Calculations

Glomerular filtration rate, single-nephron glomerular filtration rate and the deliveries of Na and K to each collection site in the nephron were calculated by standard methods as described previously (Walter & Shirley, 1986).

All results are presented as means ± s.e.means. Statistical comparisons between groups were made by means of Student's unpaired *t* test, and changes within groups were assessed by means of Student's paired *t* test.

Results

Metabolism cage experiments

The rate of saline drinking in control animals remained low throughout their period in metabolism cages (Figure 1). When hydrochlorothiazide was added to the food, rats on a normal potassium intake (group 2) increased their saline drinking up to a maximum of approximately 10 mmol $24\,h^{-1}$. Those on the high potassium intake (group 3) also increased their saline drinking in response to thiazide, but to a much smaller extent. These experiments therefore confirmed the inhibitory effect of a raised potassium intake on the sodium requirement induced by hydrochlorothiazide administration (Olesen, 1982).

Figure 2a shows the urinary sodium excretion rates in the three groups of rats; these were somewhat higher than the rates of saline ingestion due to the sodium in the food. Potassium excretion rates are shown in Figure 2b. Before the administration of hydrochlorothiazide, potassium excretion rates in groups 1 and 2 were similar to one another, whilst potassium excretion in group 3 animals was approximately 6 fold higher, reflecting the greater potassium intake. Hydrochlorothiazide caused a significant increase in potassium excretion on day 1 in group 2 animals ($P < 0.05$; paired *t* test; comparison with mean of control period), and on days 2 and 3 in group 3 animals ($P < 0.01$).

During the period of thiazide treatment, body weights increased by an average of 25 g in each of

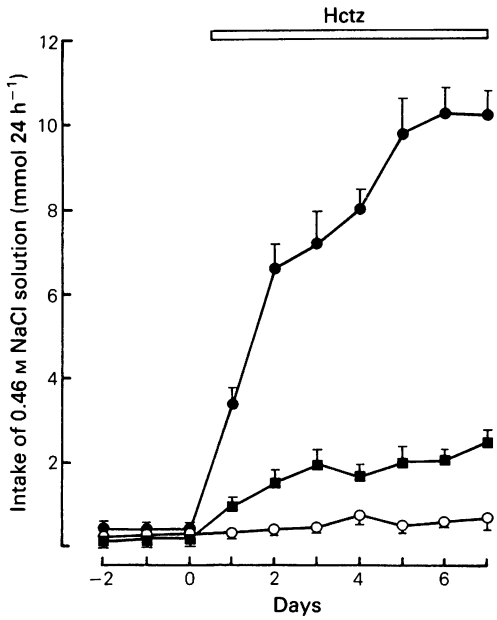

Figure 1 The effect of hydrochlorothiazide (Hctz) on the consumption of 0.46 M NaCl solution by rats on a normal or high-potassium diet. Group 1 animals (○, controls) were maintained on a diet with a potassium content of 60 mmol kg^{-1} dry weight and received no drug; group 2 rats (●) were maintained on a diet with a potassium content of 60 mmol kg^{-1} dry weight and received hydrochlorothiazide in the food at a concentration of 35 mg kg^{-1} dry weight from day 0; group 3 rats (■) were maintained on a diet with a potassium content of 360 mmol kg^{-1} dry weight and also received hydrochlorothiazide in the food at a concentration of 35 mg kg^{-1} dry weight from day 0. There were 12 rats in each group. Values are means and vertical lines indicate s.e.means.

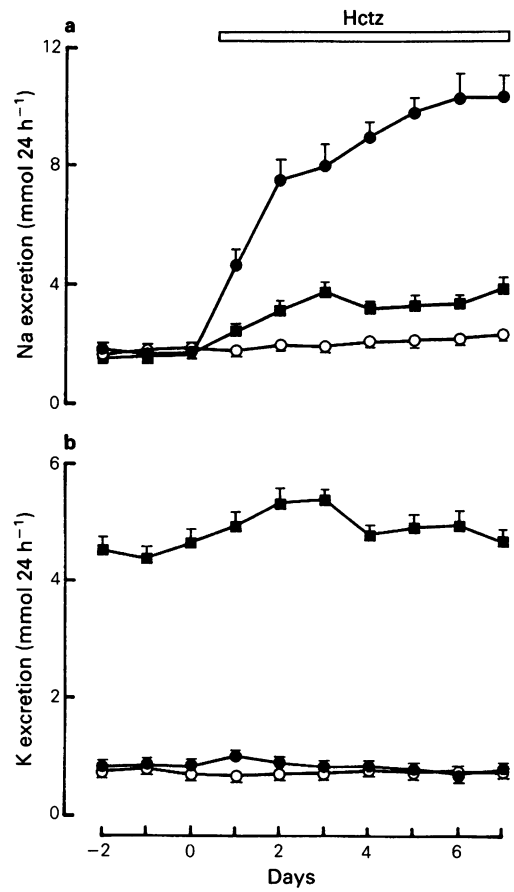

Figure 2 The effect of hydrochlorothiazide (Hctz) on (a) sodium and (b) potassium excretion rates in rats on a normal or high-potassium diet. All rats had access to 0.46 M NaCl solution. Group 1 (○); group 2 (●); group 3 (■) (groups 1–3 are explained in the legend to Figure 1). There were 12 rats in each group. Values are means and vertical lines indicate s.e.means.

groups 2 and 3 (from 243 ± 4 to 268 ± 5 g in group 2 rats; from 249 ± 5 to 274 ± 6 g in group 3 rats). In control animals (group 1) during the equivalent period body weight increased by 27 g (from 249 ± 3 to 276 ± 3 g).

Micropuncture experiments

Overall renal function, as well as single-nephron filtration rates, during the 3 h micropuncture period are shown in Table 1. There were no significant differences between the three groups with respect to either total glomerular filtration rate (GFR) or single-nephron GFR. In group 2 animals sodium excretion was considerably higher, and potassium excretion somewhat lower, than corresponding values in control (group 1) rats, whereas in group 3 animals sodium excretion was lower, and potassium excretion higher, than the control figures.

Table 2 shows the amounts of sodium and potassium delivered to the three sampling sites in superficial nephrons. In control animals, sodium delivery to the end of the proximal convoluted tubules and to the early and late regions of the distal tubule was approximately 45%, 8% and 3% of the filtered load, respectively. Sodium deliveries to the end of the proximal convoluted tubule and to the start of the distal tubule were similar in all three groups. However, the amount of sodium delivered to the late distal tubule in group 2 rats was significantly higher than

Table 1 Glomerular filtration rate (GFR) and the excretion of sodium and potassium during micropuncture

	Group 1 (control)	*Group 2 (Hctz)*	*Group 3 (Hctz + high K diet)*
GFR (ml min^{-1})	1.43 ± 0.04	1.42 ± 0.04	1.35 ± 0.04
Single-nephron GFR (nl min^{-1})	41.8 ± 1.0	44.0 ± 0.9	42.2 ± 1.4
Na excretion (μmol min^{-1})	0.51 ± 0.08	1.58 ± 0.13†	0.33 ± 0.05*
K excretion (μmol min^{-1})	0.73 ± 0.04	0.50 ± 0.03†	1.53 ± 0.10†

Results are given as means ± s.e.means. Values are for the left kidney only and are expressed per g kidney weight. There were 12 rats in each group. The number of measurements of single-nephron GFR in each group was 42–46; these values were determined from distal tubular collections only. Hctz: hydrochlorothiazide. Groups 1–3 are fully explained in the legend to Figure 1.
* $P<0.05$; † $P<0.001$ compared to the corresponding control value.

that in control (group 1) animals. In contrast, end-distal sodium delivery in group 3 animals, which had also received thiazide, was not elevated.

Potassium delivery to the end of the proximal convoluted tubule was significantly lower in each of the thiazide-treated groups (2 and 3) than in control rats. This did not result from an increase in fractional reabsorption of potassium by the proximal tubules, but from a reduction in the concentration of potassium in the plasma, and therefore in the glomerular filtrate, in thiazide-treated rats (see later). Thus the tubular fluid:plasma concentration ratios for potassium in the proximal tubule were very similar in all three groups. The differences in potassium delivery between the groups had disappeared by the start of the distal tubule.

Although in rats of groups 1 and 2 there was little evidence of significant potassium secretion into the distal tubule, in group 3 animals (which had been on a high potassium intake) nearly twice as much potassium was present at the late distal tubular collection site than had arrived at the early distal tubule. Thus end-distal potassium delivery in group 3 rats was equivalent to approximately 18% of the filtered load, as compared with 11% of the filtered load in each of the other two groups.

A blood sample was taken at the end of each experiment for measurement of packed cell volume and analysis of plasma. The results, shown in Table 3, indicate similar packed cell volumes and plasma concentrations of protein and sodium in the three groups of animals. In contrast, in each group of thiazide-treated rats there was a significant reduction in plasma potassium concentration, although the plasma potassium of group 3 rats was slightly greater than that of group 2 ($P<0.05$). Plasma aldosterone

Table 2 Sodium and potassium deliveries to each nephron segment

		Group 1 (control)	*Group 2 (Hctz)*	*Group 3 (Hctz + high K diet)*
Sodium (pmol min^{-1})	Late proximal	3070 ± 90	3096 ± 90	2975 ± 119
	Early distal	505 ± 20	452 ± 32	472 ± 28
	Late distal	158 ± 14	232 ± 19*	150 ± 16
Potassium (pmol min^{-1})	Late proximal	89 ± 3	75 ± 3*	75 ± 3*
	Early distal	15 ± 1	15 ± 1	14 ± 1
	Late distal	17 ± 1	16 ± 1	25 ± 2†

Values (means ± s.e.means) are expressed per g kidney weight. For each group the number of collection from each site was 20–24. Hctz: hydrochlorothiazide. Groups 1–3 are fully explained in the legend to Figure 1.
* $P<0.01$; † $P<0.001$ compared to the corresponding control value.

Table 3 Packed cell volumes (PCV) and plasma analysis

	Group 1 (control)	*Group 2 (Hctz)*	*Group 3 (Hctz + high K diet)*
PCV (%)	48.3 ± 0.6	47.9 ± 0.3	48.3 ± 0.5
Plasma protein ($g l^{-1}$)	51.6 ± 1.1	52.3 ± 2.4	52.0 ± 1.5
Plasma sodium ($mmol l^{-1}$)	144 ± 1	143 ± 1	145 ± 1
Plasma potassium ($mmol l^{-1}$)	3.92 ± 0.05	3.36 ± 0.05*	3.54 ± 0.06*
Plasma aldosterone ($pmol l^{-1}$)	1565 ± 98	628 ± 92*	1557 ± 150

Results are given as means ± s.e.means. There were 12 rats in each group; plasma aldosterone was measured in only 6 animals per group. Hctz: hydrochlorothiazide. Groups 1–3 are fully explained in the legend to Figure 1.
* $P < 0.001$ compared to the corresponding control value.

concentration, measured in six rats from each group, was significantly lower in group 2 animals than in either of the other groups.

Discussion

It has been demonstrated previously that rats will drink significant quantities of 0.46 M NaCl solution only if made sodium deficient (Richter, 1936; Olesen & Thomsen, 1981). The amount of saline drunk is then an estimate of the sodium requirement of the animal. In the present experiments sodium excretion, and therefore saline drinking, was increased markedly during hydrochlorothiazide treatment in group 2 rats, reaching a maximum value after about 5 days. Thus the availability of 0.46 M NaCl solution prevented the 'braking effect' normally observed during chronic thiazide treatment, whereby sodium excretion returns to normal levels as a result of compensatory changes in renal function (Steven & Skadhauge, 1969; Walter & Shirley, 1986). Prevention of such compensatory changes was desirable in the present study in order to facilitate examination of the influence of potassium on the action of thiazides.

When the dietary potassium content was raised from 60 to 360 $mmol kg^{-1}$ dry weight (group 3 rats), hydrochlorothiazide had much less effect on sodium excretion and saline drinking. Our results therefore support Olesen's (1982) contention that a high potassium intake blunts the natriuretic effect of thiazides. In view of reports that a raised potassium intake can cause a small increase in sodium excretion (Keith & Binger, 1935; Olesen & Thomsen, 1981), it might be argued that group 3 rats were in a sodium-depleted state before the administration of thiazide (since these animals were placed on the high potassium diet from the first day in metabolism cages), which could be offered as an explanation of the reduced response to the diuretic. However, this possibility is unlikely for two reasons. First, 0.46 M saline was available throughout, so that any sodium deficit could have been corrected. Secondly, a separate series of experiments was performed in which the extra potassium was added to the food only from the start of hydrochlorothiazide administration. The results were identical to those in group 3 rats, i.e. the intake of 0.46 M NaCl increased to approximately 2 $mmol day^{-1}$ (compared with approximately 10 $mmol day^{-1}$ in thiazide-treated rats on a normal potassium diet).

It should be emphasized that the effect of a raised potassium intake on saline drinking in thiazide-treated rats is a *result*, rather than the *cause*, of the reduced natriuresis: Olesen (1982) has shown that a raised potassium intake reduces the natriuretic effect of hydrochlorothiazide even in rats which do not have access to saline.

In order to investigate the renal mechanisms responsible for the effect of potassium, animals were anaesthetized and micropuncture experiments were performed. To ensure that plasma hydrochlorothiazide concentrations during the micropuncture studies would be at effective levels, the day/night cycle of the animals was adjusted so that each micropuncture experiment took place shortly after the main feeding period. A difficulty faced over the experimental protocol was in setting suitable infusion rates for the three groups of animals. It could be argued that the infusion rates should have matched the sodium excretion rates measured before anaesthetization of the animals. However, we elected to use the same infusion rate for all three groups in order to prevent the possibility of forcing an 'acute' natriuresis in group 2 rats by saline loading. Despite this, during micropuncture the sodium excretion of group 2 rats was still 3–4 times higher than values in the other two groups, although in all animals values were somewhat lower than in the conscious state, as commonly found in anaesthetized, surgically-operated rats (Maddox *et al.*, 1977). For reasons not understood, the sodium excretion of group 3 rats was slightly lower than that of control animals. Potassium excretion during

micropuncture was considerably higher in group 3 rats (i.e. those which had been on the high potassium intake) than in the other groups. Unexpectedly, however, the potassium excretion of group 2 rats was somewhat lower than that of controls.

Thus, although in general the sodium and potassium excretion rates during micropuncture reflected those seen when the rats were in metabolism cages, some (relatively minor) exceptions were observed, indicating an effect of the micropuncture set-up itself and underlining the need for caution when extrapolating from micropuncture results to the situation in conscious animals.

No differences were observed between groups with respect to total glomerular filtration rate or single-nephron filtration rate. Absolute and fractional reabsorption by the proximal convoluted tubule was also similar in all three groups, amounting to approximately 55% of the filtrate. When rats are treated chronically with thiazide, but not allowed access to saline, the ensuing sodium depletion leads to an increase in fractional reabsorption by the proximal tubules (Walter & Shirley, 1986). Clearly the availability of saline prevented this from happening.

Deliveries of sodium to the start of the distal tubule were also similar in all three groups of rats. However, at the late distal tubule sodium delivery was elevated in group 2 rats, the result, presumably, of an inhibitory action of hydrochlorothiazide on sodium reabsorption in the distal tubule; such an effect has been demonstrated previously in acute studies in which distal tubules were microperfused with chlorothiazide (Costanzo, 1985; Velazquez & Wright, 1986). This inhibition of distal tubular sodium reabsorption was not seen in group 3 animals. It seems clear from the present study, therefore, that the blunting of the thiazide-induced natriuresis in rats on a high-potassium intake results from an effect which is located in the distal tubule itself, rather than from changes either in glomerular filtration or in sodium reabsorption elsewhere in the nephron.

The issue which remains to be addressed is how a raised potassium intake prevents the thiazide-induced inhibition of sodium reabsorption in the distal tubule. It could result either from a direct antagonistic effect of potassium on hydrochlorothiazide's action or from a compensatory increase in sodium reabsorption at or beyond the site of action of the thiazide. Since little is known about the cellular mechanism of action of thiazides (Odlind, 1984) it is difficult to comment on the first possibility, although the fact that plasma potassium levels were little different from those of group 2 rats may cast some doubt on it. Concerning the second possibility, there are at least two potential explanations. The high excretion rates of potassium in group 3 rats resulted from increased potassium secretion in the distal tubule and possibly beyond it. If this potassium secretion were obligatorily linked with sodium reabsorption, this might offer an explanation for the blunting of the thiazide-induced natriuresis. The cause of the high rate of potassium secretion in these animals is not clear. Plasma aldosterone levels and distal tubular fluid flow rates were similar to values in control animals, while the plasma potassium concentration was reduced.

A second reason for distal tubular sodium reabsorption being greater in group 3 rats than in those of group 2 might have been the differences in their respective aldosterone levels. Whilst plasma aldosterone concentrations in group 3 rats were similar to those of controls, those of group 2 animals were consistently lower. Although it was anticipated that the stimulation of the renin-angiotensin-aldosterone system which usually follows thiazide administration would not occur in the present study, owing to the prevention of extracellular volume contraction, a *reduced* plasma aldosterone concentration in group 2 rats was unexpected. It could be argued that these animals had in fact expanded their extracellular volume by drinking saline in excess of their requirements. Although this possibility could not be assessed directly by determining sodium balances, since faecal sodium losses were unknown, it seems an unlikely explanation for several reasons. The increases in body weight of group 2 rats during hydrochlorothiazide administration were not greater than those of control animals. Furthermore, packed cell volumes and plasma protein concentrations, indirect indices of extracellular volume, were very similar in group 2 rats to values in each of the other groups. Finally, a few determinations of plasma renin activity were made, and these did not indicate suppression of the renin-angiotensin system in group 2 animals. Results ($n = 4$ for each group) were 3.1 ± 1.0, 3.2 ± 1.4 and 1.9 ± 0.5 ng angiotensin I ml^{-1} plasma h^{-1} in groups 1, 2 and 3 respectively.

Whatever the explanation for the reduced plasma aldosterone levels in group 2 animals, the fact that no such reduction was observed in hydrochlorothiazide-treated rats on a high potassium intake (group 3) provides a possible explanation for the blunting of the thiazide-induced natriuresis. In separate experiments we tried to assess the role of aldosterone by adding spironolactone to the food of all three groups of rats. The findings were essentially unchanged (i.e. hydrochlorothiazide induced a marked natriuresis which was blunted by a high potassium intake), implying that differences in aldosterone levels did not contribute to the different responses of group 2 and group 3 animals. However, it should be emphasized that in our hands spironolactone administration interfered only minimally with the sodium-retaining effects of acute injections of deoxycorticosterone acetate or aldosterone. Other investigators have also found sodium

excretion by the rat kidney to be resistant to the administration of spironolactone (Adam & Adams, 1985).

Thus the precise mechanism by which a high potassium intake interferes with the natriuretic effect of hydrochlorothiazide has yet to be identified. However, in view of the fact that potassium supplements are commonly used in an attempt to combat thiazide-induced hypokalaemia, these observations in rats, if applicable to man, might be of some clinical significance inasmuch as the therapeutic actions of thiazides are considered to rely at least partly upon their natriuretic effect. Although it is recognised that potassium supplements given to patients do not usually increase the total potassium intake by more than two fold, whereas in the present experiments it was increased six fold, the latter almost abolished the natriuretic effect. In unpublished studies we have found much smaller increases in potassium intake to have a significant inhibitory effect on the natriuresis. A second problem with potassium supplements concerns their relatively poor ability, compared with potassium-sparing diuretics, to diminish the reduction in plasma potassium (Ramsay *et al.*, 1980; Jackson *et al.*, 1982). The present results show that in a situation where sodium depletion is prevented, potassium supplements have only a very minor effect on the thiazide-induced hypokalaemia.

In summary, the effect of a raised potassium intake in blunting the natriuresis induced by hydrochlorothiazide has been confirmed. This effect is located in the distal tubule and occurs as a result of interference with the inhibitory action of the diuretic on sodium reabsorption in this region of the nephron.

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