water. The intracellular reservoir presumably contributes the 'extra' fluid under the stimulus of adrenal glucocorticoid action which apparently is concerned in some unknown manner with active transfer of Na and water across cell membranes. The hormone may perhaps serve to prime the sodium water pump of higher organisms.

Summary. Adrenalectomized dogs deprived of food for 48 and water for 24 hours respond to injections of prednisolone by as marked an increase in plasma volume as observed in similarly fasted intact dogs. Desoxycorticosterone when given simultaneously with the glucocorticoid does not potentiate the plasma volume-raising action of the latter. Massive doses of prednisolone (315 mg) induced striking elevation of the plasma volume of dogs fasted 72 hours and deprived of water for 48 hours, despite excretion of 740 ml of fluid as urine. Blood sugar and plasma Na also increased but the hemoglobin and hematocrit fell sharply. Fasted but non-injected adrenalectomized dogs reacted quite differently. They exhibited drastic decline in plasma volume and blood sugar whereas hemoconcentration increased. The fluid mobilized to increase the plasma volume apparently was shifted from the intracellular compartment under influence of the hormone which is somehow concerned with active transfer of sodium and water across cellular membranes.

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Effect of Urea-Saline Diuresis on Renal Clearance of Calcium, Magnesium, and Inorganic Phosphate in Man.* (30839)

O. S. BETTER,† H. C. GONICK,‡ L. C. CHAPMAN, P. D. VARRADY, C. R. KLEEMAN (Introduced by M. H. Maxwell)

Cedars-Sinai Medical Research Institute, and Division of Medicine, Cedars-Sinai Medical Center, and Department of Medicine, University of California School of Medicine, Los Angeles

In a concurrent study(1) on the renal handling of divalent ions in a large group of patients with chronic renal failure, it was found that there was a close correlation between the degree of renal insufficiency and the progressive rise in the clearance ratio $\frac{C_{\text{Divalent ion}}}{C_{\text{Creatinine}}}$. It is apparent that in chronic renal failure there is an increasing osmotic load per nephron as renal mass decreases, and as it has been demonstrated in dogs that osmotic diuresis augments the renal clearance of calcium and magnesium(2-5) it is reasonable to conclude that the high osmotic load per nephron in chronic renal disease contributes significantly to the rising clearance ratio of calcium and magnesium. The present experiment was designed to produce an osmotic diuresis in the normal human similar in magnitude and content to that sustained by the uremic patient so that the resultant changes in calcium and magnesium excretion at similar osmotic loads per nephron could be compared. GFR, as measured by inulin clearance,

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† Presently at Rambam Hospital, Haifa, Israel.

‡ Medical Service, Wadsworth Hospital, Veterans Admin.

was taken as a reasonable estimate of functioning nephron mass. The validity of this assumption has recently been discussed(6).

Methods. Five normal male volunteers ranging in age from 20-37 years served as the experimental population. Inulin clearance was performed according to Smith(7). A solution of urea and saline was made by adding 90 g of urea (as Urevert® Travenol) to 1000 ml normal saline. This solution was infused independently of inulin at a rate designed to provide a total of 1.5 g urea/kg body weight over a period of 2.5 hours. The experiments were conducted in mid-morning in the fasting state. The subjects lay supine during the infusion and stood only to void. Urine was collected (by voiding) at 20 minute intervals. Mid-point heparinized venous blood samples were obtained between collections, centrifuged and the plasma separated. Two control collections were obtained after equilibration of inulin and prior to urea saline infusion. Water losses were replaced per os. Four additional young males were infused with 0.45% saline at a rate of 1.5-3 ml/min over the same period of the day as the previous 5 subjects. This served as control for possible diurnal variation in the clearance of calcium, magnesium, and phosphate. Urine and plasma samples were assayed for inulin (7), total osmolarity (Fiske osmometer), sodium (flame photometry), inorganic phosphate(8), calcium(9), and magnesium(10). Plasma diffusible calcium, magnesium and inorganic phosphate were determined on 2 samples for each infusion experiment. One sample was obtained prior to and the second just before the end of the urea saline infusion. Ultrafiltration was performed at room temperature using the Lavietes anaerobic ultrafiltration technique(11). The average per cent diffusibility was used to calculate diffusible divalent ions for all plasma specimens. Water content of plasma was determined by refractometry (Goldberg Refractometer, American Optical Co., Buffalo, N. Y.). Plasma ionized calcium was measured by a micromodification(12) of the double-wave length spectrophotometry method of Ettori and Scoggan(13). All clearances of divalent ions are expressed in terms of their diffusible fraction.

TABLE I. Maximal Solute Excretion and Minimal Urine/Plasma (U/P) Inulin Ratio Attained in Each Subject During Urea-Saline Infusion.

Subject	Maximal Uosm × V (mOsm/min)	Minimal U/P inulin ratio
OB	4.3	8.1
MJ	8.2	4.2
DC	7.5	3.3
AC	5.4	8.4
RR	7.2	5.9

Results. The magnitude of the osmotic diuresis induced by urea saline infusion is presented in Table I. On the average, the solute excretion rate was approximately 6- to 7-fold the basal rate. About two-thirds of urinary solute output was contributed by urea, whereas about one-third was contributed by sodium chloride. Changes in BUN, inulin clearance, and plasma level of calcium, magnesium, and inorganic phosphate are given in Fig. 1. During the infusion BUN rose on the average to about 100 mg/100 ml, while C_{IN} fell approximately 20%. In general, there was a slight drop in the plasma level of diffusible calcium, magnesium, and inorganic phosphate. This drop was due to a fall in total ion level rather than to change in diffusibility (which remained constant throughout in spite of a slight fall in plasma protein during the infusion). Plasma ionized calcium was measured in 2 cases before and at the end of the infusion. There was no change in the ratio ionized Ca/total Ca in these 2 cases. Fig. 2 illustrates the correlation between sodium excretion rate and the clearance of calcium and magnesium and between the clearances of calcium and magnesium plotted against each other (Fig. 2). There was a linear relationship between each pair of these 3 parameters over the range of solute excretion studied. The slopes of the regression lines of C_{Ca}/C_{IN} and C_{Mg}/C_{IN} on sodium excretion rate were 4.36 and 4.34 respectively. This difference in slopes was not statistically significant (Table II). For any given level of sodium excretion rate, C_{Mg}/C_{IN} was higher than C_{Ca}/C_{IN} by approximately the difference in intercepts (2.75). When C_{Ca}/C_{IN}

was plotted against sodium $\left(\frac{U_{Na}V}{P_{Na}}\right)$ the slope of the regression line was 0.6. When clearance of magnesium was plotted against the clearance of calcium the slope of the regression line was 0.41. The relationship among the 3 variables (sodium excretion rate, calcium and magnesium clearances) could be described by a first order or linear equation. In contrast to the definite effect of osmotic diuresis on calcium and magnesium excretion, it did not cause any change in phosphate clearance (data not shown).

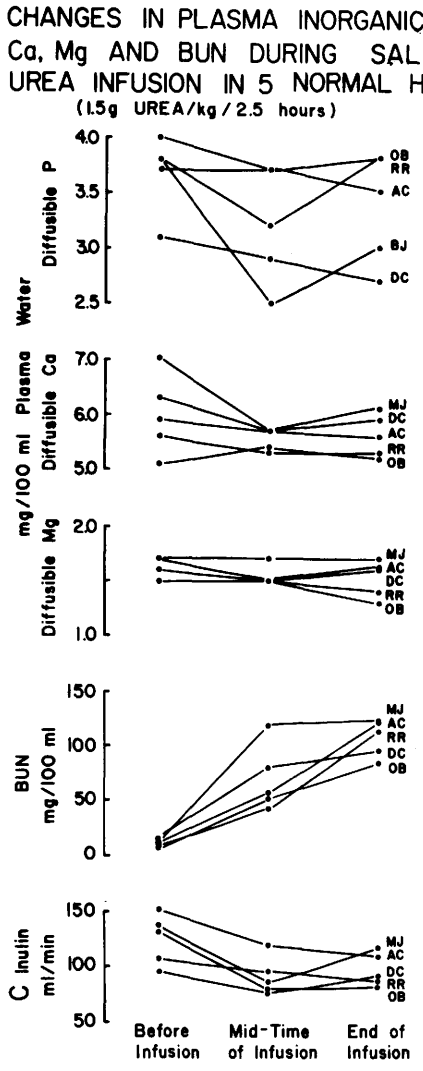

FIG. 1. Changes in plasma diffusible inorganic phosphate, calcium and magnesium, and in BUN and inulin clearance during urea-saline infusion in 5 normal humans.

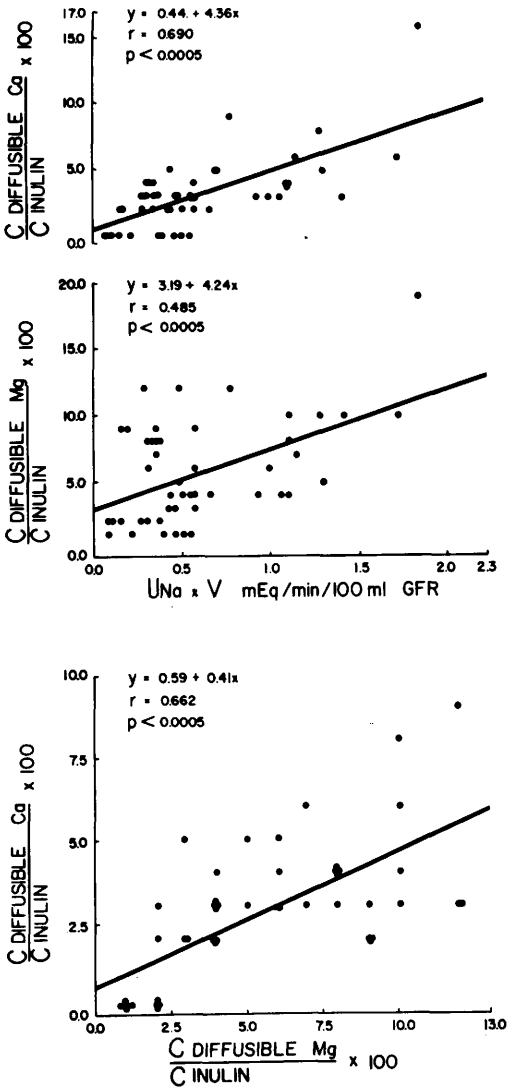

FIG. 2. Correlation between calcium and magnesium clearances and sodium excretion rate (upper 2 figures) and between clearances of calcium and magnesium (lower figure), during urea-saline diuresis in 5 normal humans.

The clearances of calcium, magnesium, and inorganic phosphate (all divided by C_{IN}) were plotted against time in the 4 control subjects. No consistent pattern was noted (probably due to the shortness of the experiment, the correction of the clearances for 100 ml GFR and the fact that the subjects

TABLE II. Relation of Calcium and Magnesium Clearances to Sodium Excretion Rate. Comparison of correlation in the present study with the two previous reports.

Author	Diuretic agent	Species	Slope of regression line $C_{Ca}/U_{Na} \times V$	Slope of regression line $C_{Mg}/U_{Na} \times V$
Wesson(4)	Urea	Dog	3.2	3.0
Kupfer and Kossovsky(5)	Mannitol	"	3.8	4.0
Present study	Urea-saline	Human	4.4	4.2

were inactive and in the fasting state during the study).

Fig. 3 compares the relationship between sodium excretion rate and calcium clearance after high sodium ingestion reported previously from this laboratory(14), with the same relationship during urea saline infusion. At comparable rates of sodium excretion calcium clearance was at least as high (and in 3 out of 6 cases higher) in the group that ingested salt than in the group that had the urea saline infusion. Correction of the clearances for 100 ml GFR did not change this conclusion. In the individual experiments (subjects O.B., M.J., D.C., A.C., R.R.) in the present study the changes in clearance of magnesium followed closely those of calcium.

Discussion. The maximal rise in BUN and solute excretion rate per unit of filtration rate (5 mOsm/min/100 ml GFR) were comparable to values seen in moderately advanced renal failure. A higher solute output could not be attained due to emergence of such symptoms as severe headache and dizziness when the subjects stood to void. The salient feature of the present study is the fact that urea saline diuresis in man increases the clearances of calcium and magnesium but does not affect the clearance of phosphate. This is in agreement with previous observa-

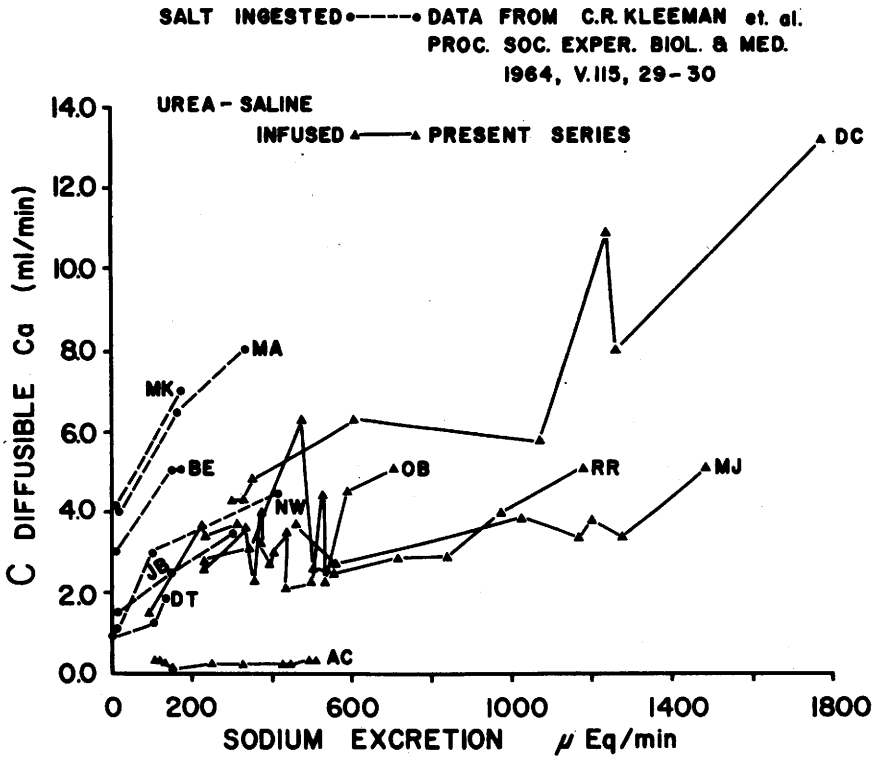

FIG. 3. Comparison between effects of high salt ingestion(14) (dashed line) and urea-saline infusion (solid line), on relation between calcium clearance and urinary sodium excretion rate.

tions on the osmotic diuresis in the dog(2-5). Within the range of solute diuresis reached, sodium excretion rate and calcium and magnesium clearances were directly related. The relationship between any two of these parameters could be represented by a straight line (Fig. 2). The relationship among all 3 variables was represented by a linear plane computed by the method of multiple regressions. The formula relating all 3 variables was a first order equation:

$$Y = 1.32 + 0.0032\,X + 0.0068\,Z$$

where $Y = C_{Ca}/C_{IN} \times 100$, $X = C_{Mg}/C_{IN} \times 100$, $Z = U_{Na} \times V/100$ ml GFR (Na excretion expressed in μEq/min). The direct relationship between the clearances of calcium and sodium excretion rate support Walser's (3) and Wesson's(4) suggestion that sodium, calcium and magnesium reabsorption shared a common pathway. The lack of effect of osmotic diuresis on phosphate excretion noted in the present study has been repeatedly demonstrated in dogs(4,5,15).

The slopes of the regression lines of C_{Ca} and C_{Mg} on sodium excretion rate observed in this study did not differ significantly from one another and were in fairly good agreement with two previous reports on dogs (Table II) in spite of the differences in species and methods employed. The calculated slope of the regression line of the clearance of diffusible calcium on sodium clearance was 0.62. This differs from Walser's(3) slope of approximately 1 for the same relationship. The difference in slopes between our and Walser's study may be explained in part by differences in methods (Walser's dogs were on low sodium regimen and received chlorthiazide up to one day prior to the experiments), and by the small sample of normal subjects studied. It should be emphasized that the relationship found between the clearances of calcium and magnesium and the rate of sodium excretion reported in this paper was demonstrated within relatively stable experimental conditions. Pathologic or toxic changes may disrupt this relationship as has been shown, for instance, for organic mercurials(4) and digitalis(5). Comparison of the calciuric effect of high salt ingestion with the calciuric effect of urea saline infusion reveals that the former is at least as effective in promoting calcium excretion (relative to $U_{Na} \times V$). This is in accord with Wesson's observation(4) that urea diuresis has little calciuric effect.

Summary. Urea saline diuresis in 5 normal humans induced a linear increase in the clearance of calcium and magnesium and had no effect on the clearance of inorganic phosphate. The relationship among all 3 variables (clearances of calcium and magnesium and excretion rate of sodium) could be expressed by a first order equation. The data presented are in accord with previous reports on the effect of osmotic diuresis on divalent ion excretion in dogs.

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