

Spontaneous Variations in Electrolyte Excretion in the Awake Dog^{1,2} (37868)

DAVID L. HARTENBOWER,³ ROBERT M. FRIEDLER,
 JACK W. COBURN, AND SHAUL G. MASSRY
 (Introduced by Alvin Sellers)

*Medical and Research Services, Veterans Administration Wadsworth Hospital Center,
 and Renal and Hypertension Service, Medical Research Institute and Department
 of Medicine, Cedars-Sinai Medical Center, and Department of Medicine,
 UCLA School of Medicine, Los Angeles, California*

Considerable attention has been directed toward the diurnal variation in the pattern of renal excretion of electrolytes in man (1-3). The nature and characteristics of patterns of excretion in other mammals have not been well-characterized. When studies of renal physiology are carried out, it is often assumed that alterations in the excretion rates from one period of time to another occur only as a result of manipulative procedures involved. The dog is commonly used as a laboratory model for experiments in renal physiology, and the present experiments were undertaken to characterize the normal pattern of renal function and the rates of excretion of electrolytes in the unanesthetized dog during hours of the day. No attempt was made to evaluate changes during the full 24-hr period. As parathyroid hormone would be expected to influence the renal handling of phosphorus and calcium, additional studies were undertaken in parathyroidectomized dogs.

Materials and Methods. Experiments were carried out in 14 adult female mongrel dogs, weighing 16-22 kg, which had previously been subjected to episiotomy. The dogs had been conditioned to spending prolonged

periods in the laboratory either standing loosely supported in a frame or lying on a table with loose restraints. For at least 2 weeks before the study, the dogs received a constant diet of 454 g of the same standard canned dog food (Friskies, Carnation Co., Los Angeles, CA) at 8-9 AM daily except the day of the study. Water was allowed *ad lib.* up to the time the study began.

Thyroparathyroidectomy was carried out in 6 dogs at least 2 weeks prior to study. Hypocalcemia was documented at the time of the study as evidence for the adequacy of parathyroidectomy. Thyroid extract, 65 mg, was given orally three times a week to prevent hypothyroidism. Supplemental calcium, as oral calcium gluconate, was given in doses of 10 g/day to dogs having serum calcium levels below 6 mg/100 ml; iv calcium gluconate, 1 g/dose, was given to dogs manifesting tetany. No calcium supplement was given on the day prior to the study.

Clearance studies were carried out between 8 AM and 8 or 9 PM with the dogs in the awake state loosely supported in a canvas sling. Priming and sustaining solutions of creatinine were given into a leg vein via an indwelling plastic catheter. To avoid the effects of NaCl, glucose, or appreciable volumes of infusate, the sustaining solution contained only creatinine, 15 g/liter, and was given by a constant infusion pump at 0.4 ml/min. Urine samples were collected through an indwelling bladder catheter at hourly intervals; adequate bladder emptying was assured by air rinse and mild suprapubic

¹ Presented in part before the 57th Annual Meeting of the Federation of American Societies for Experimental Biology, April 15, 1973, Atlantic City, NJ.

² Supported in part by USPHS Grants AM 5383, AM 5630, and RR 5468.

³ Research and Education Associate of the Veterans Administration.

pressure. Venous blood samples were obtained at the midpoint of each collection period.

Creatinine, sodium, potassium, phosphorus, calcium, and magnesium were determined in plasma and urine utilizing methods previously reported from this laboratory (4), and the clearances of diffusible calcium and magnesium were calculated from values of ultrafilterable calcium and magnesium in a manner described earlier (5).

Results. A summary of all experiments in dogs with intact parathyroid glands is given in Table I. Urine volume and the excretion rates of sodium, calcium, magnesium, phosphorus, and potassium rose to peak values and later declined. The excretion of each electrolyte followed a similar pattern from one animal to another, although the absolute quantity excreted and the amplitude of change varied considerably. To adjust for variations in quantities excreted from one animal to another, the excretion rates are calculated as the percent of the mean value observed over the entire 12- or 13-hr period of study for each individual dog. The means for all animals, expressed as the percent of the filtered load excreted for each ion except potassium, are shown in Fig. 1.

Glomerular filtration rate (GFR) showed a slight rise in the morning with an increase from 9–10 AM to 11 AM–noon of 12.4 ± 5.7 ml/min (mean \pm SE), $P = .05$; GFR then remained relatively stable until 4 PM; between 5 and 7 PM, GFR values were significantly lower than that observed at 11 AM–noon ($P < .05$). Serum levels for sodium, potassium, calcium, magnesium, and phosphorus did not change significantly. Urinary sodium excretion increased by a mean of $69 \mu\text{Eq}/\text{min}$ from the value at 8–9 AM with the peak value noted between noon and 1 PM. The maximum change in an individual dog was from $3 \mu\text{Eq}/\text{min}$ (10–11 AM) to $221 \mu\text{Eq}/\text{min}$ (noon–1 PM). The fractions of filtered calcium and magnesium followed somewhat similar patterns with peak values occurring between 10 AM and noon, earlier than that observed for sodium. The mean increase in calcium excretion between 8–9 AM and 10–11 AM was $10 \mu\text{g}/\text{min}$; but one animal showed an increase from 3 to $65 \mu\text{g}/$

TABLE I. Daytime Pattern of Urine Flow, Glomerular Filtration Rate (GFR), and Electrolyte Excretion in 8 Awake Normal Dogs.^a

Clock time	8-9	9-10	10-11	11-noon	Noon-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
Urine flow (ml/min)	0.43 ± 0.16	0.36 ± 0.11	0.53 ± 0.18	0.62 ± 0.16	0.68 ± 0.17	0.54 ± 0.13	0.55 ± 0.14	0.43 ± 0.11	0.35 ± 0.082	0.24 ± 0.053	0.27 ± 0.059	0.22 ± 0.037	0.29 ± 0.033
GFR (ml/min)	73 ± 12	63 ± 6	71 ± 9	76 ± 8	72 ± 7	71 ± 8	68 ± 5	62 ± 6	63 ± 7	60 ± 4	59 ± 4	65 ± 6	66 ± 1.4
U_{NaV} ($\mu\text{Eq}/\text{min}$)	8.5 ± 2.2	22 ± 9	28 ± 8	61 ± 21	77 ± 24	57 ± 13	51 ± 11	36 ± 8	22 ± 5	13 ± 4	11 ± 4	8 ± 3	14 ± 7
U_{CaV} ($\mu\text{g}/\text{min}$)	12 ± 2.0	16 ± 5.6	22 ± 6.9	21 ± 5.2	16 ± 7.2	6.7 ± 1.0	4.3 ± 0.7	3.3 ± 0.5	2.7 ± 0.7	2.3 ± 0.5	2.9 ± 0.7	2.4 ± 0.6	2.8 ± 0.6
U_{MgV} ($\mu\text{g}/\text{min}$)	44 ± 14	36 ± 7	46 ± 12	63 ± 12	43 ± 10	29 ± 5	25 ± 6	18 ± 4	17 ± 4	16 ± 3	16 ± 3	16 ± 4	19 ± 7
U_{PV} ($\mu\text{g}/\text{min}$)	65 ± 31	150 ± 86	210 ± 150	202 ± 123	155 ± 79	379 ± 127	458 ± 145	406 ± 96	365 ± 99	274 ± 83	228 ± 77	162 ± 75	255 ± 121
U_{KV} ($\mu\text{Eq}/\text{min}$)	25 ± 8.9	31 ± 5.7	36 ± 6.9	39 ± 6.9	42 ± 7.8	34 ± 5.4	30 ± 8.2	23 ± 4.6	19 ± 3.0	15 ± 1.8	13 ± 1.4	11 ± 1.4	11 ± 1.9

^a All data indicate mean \pm SE. U_{NaV} = sodium excretion; U_{CaV} = calcium excretion; U_{MgV} = magnesium excretion; U_{PV} = phosphate excretion; and U_{KV} = potassium excretion.

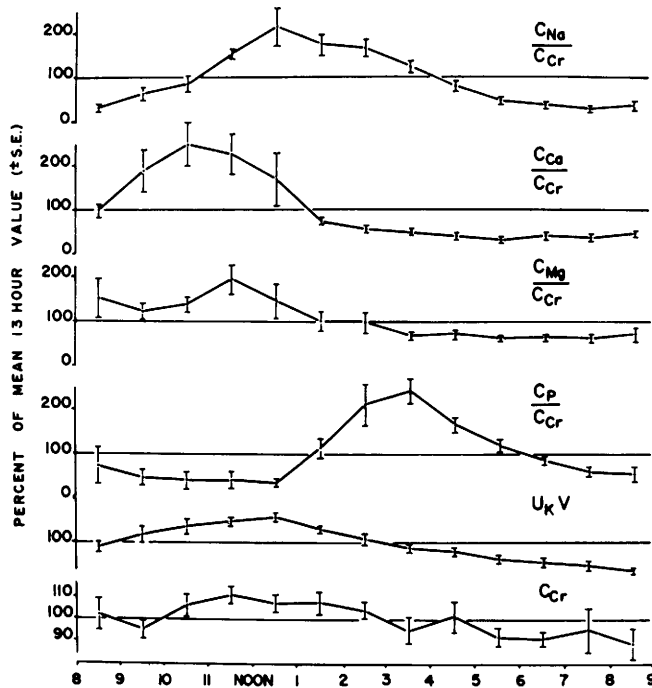


FIG. 1. Pattern of renal function and electrolyte excretion in 8 normal dogs. Excretion rates are calculated as the percent of the mean value observed in individual dogs over the entire 13-hr period of study and are expressed as the percent of the filtered load excreted for each ion except potassium.

min during the morning. The mean urinary magnesium excretion increased by $27 \mu\text{g}/\text{min}$ over the same period of time with a maximum change from 4 to $86 \mu\text{g}/\text{min}$ in one dog.

Potassium excretion showed less variability than seen with other electrolytes, but maximal values were noted between 11 AM and noon.

The most striking and consistent variation occurred with phosphorus. Urinary phosphorus excretion remained relatively constant from 8 AM until 1 PM; over the next 2 hr the mean percent of filtered phosphorus excreted increased from 4.0 to 19.5%; one animal showed an increase in phosphorus excretion of $1186 \mu\text{g}/\text{min}$ during this time.

The urinary excretion rates of the various electrolytes and the fraction of their filtered loads excreted showed no correlation with their concentrations in the serum or filtered loads.

The results of experiments performed in

thyroparathyroidectomized dogs are summarized in Table II and Fig. 2. The mean serum calcium level was $6.4 \pm .15$ (SE) $\text{mg}/100 \text{ ml}$. Glomerular filtration rate showed a different pattern than that observed in intact dogs with GFR increasing to values late in the afternoon (4–7 PM) that were significantly greater than those observed between 9 and 11 AM ($P < .02$ by paired t tests). For sodium, calcium, and magnesium, the rates of urinary excretion were relatively constant throughout the period of observation. The maximum mean increase in sodium excretion was $19 \mu\text{Eq}/\text{min}$, a value 28% of the increment observed in the intact dogs. The maximum change in sodium excretion rate in an individual dog was from $12.5 \mu\text{Eq}/\text{min}$ at 8–9 AM to $98 \mu\text{Eq}/\text{min}$ at 11 AM–1 PM.

Calcium excretion showed a mean increase of $6.7 \mu\text{g}/\text{min}$ from 8–9 AM to 11 AM–noon. The greatest change in one dog was $18.5 \mu\text{g}/\text{min}$. The smaller increment of change of calcium excretion in the thyo-

parathyroidectomized dogs may be due to the decrease in filtered load of calcium.

The peak urinary excretion of magnesium occurred between 2 and 3 PM in contrast to 11 AM-noon in the intact dogs. The mean change in magnesium excretion from 8-9 AM and 2-3 PM was 29.5 $\mu\text{g}/\text{min}$ with a maximum change from 40 to 122 $\mu\text{g}/\text{min}$ in an individual dog.

Potassium excretion remained constant until 3 PM and then decreased. The maximum mean difference was 27 $\mu\text{Eq}/\text{min}$ while one dog decreased from 81 to 32 $\mu\text{Eq}/\text{min}$.

The pattern of phosphate excretion in the thyroparathyroidectomized dogs was similar to that seen in the intact dogs. Phosphate excretion was constant until noon and then the mean percent of filtered phosphate excreted increased from 4.6 to 12.5% at 4-5 PM; one animal showed an increase of 445 $\mu\text{g}/\text{min}$ during this time.

Discussion. The present study demonstrates that substantial and significant variations in the urinary excretion rates of various electrolytes occur spontaneously in the dog during the daytime hours. These patterns of excretion were, in general, similar to those reported in man (1-3). These data emphasize the need to properly consider natural variations in electrolyte excretion when one evaluates experiments of their renal handling.

The most striking changes were observed in the excretion rates of sodium, calcium, and phosphorus. However, these changes did not parallel each other. The excretion of both calcium and sodium increased during the morning hours with calcium excretion reaching its peak at 11 AM, 2 hr earlier than the peak of sodium excretion. Thereafter, the excretion of both of these ions fell to levels below the mean excretion for the entire period of the study. In contrast, phosphorus excretion was at its nadir throughout the morning hours and then increased to reach its peak between 2 and 4 PM at the time when both calcium and sodium excretion were falling or low. This dissociation between the excretion rates of these three ions may permit one to speculate about the sites in the nephron which may be responsible for these variations. It is well-accepted that the reabsorption of sodium, calcium,

TABLE II. Daytime Pattern of Urine Flow, Glomerular Filtration Rate (GFR), and Electrolyte Excretion in 6 Awake Thyroparathyroidectomized Dogs.

Clock time	8-9	9-10	10-11	11-noon	Noon-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8
Urine flow (ml/min)	0.29±0.10	0.23±0.05	0.26±0.04	0.29±0.06	0.29±0.06	0.29±0.05	0.28±0.06	0.25±0.04	0.24±0.04	0.30±0.07	0.40±0.18	0.28±0.08
GFR (ml/min)	66±8	48±7	57±7	58±6	66±8	69±7	67±6	66±8	69±6	76±7	71±9	68±11
U _{Na} V ($\mu\text{Eq}/\text{min}$)	41±39	32±8	38±11	49±17	46±18	47±16	51±19	40±13	41±13	41±14	39±15	41±14
U _{Ca} V ($\mu\text{g}/\text{min}$)	3.8±1.3	6.2±1.0	7.6±2.7	10.5±3.6	8.9±2.5	6.7±1.9	7.7±2.9	6.1±1.9	6.3±1.9	6.1±1.9	6.9±2.0	5.5±1.4
U _{Mg} V ($\mu\text{g}/\text{min}$)	20±14	27±7	29±11	35±11	38±12	44±14	49±19	38±14	40±12	37±12	37±15	27±6
U _P V ($\mu\text{g}/\text{min}$)	192±90	166±48	169±55	190±68	215±73	341±97	453±81	499±37	562±45	486±61	432±56	427±84
U _K V ($\mu\text{Eq}/\text{min}$)	52±11	43±7.2	55±7.8	5.9±7.8	56±7.5	5.7±5.9	5.9±6.1	39±2.1	38±2.3	36±3.3	33±4.5	32±6.1

Legend same as Table I.

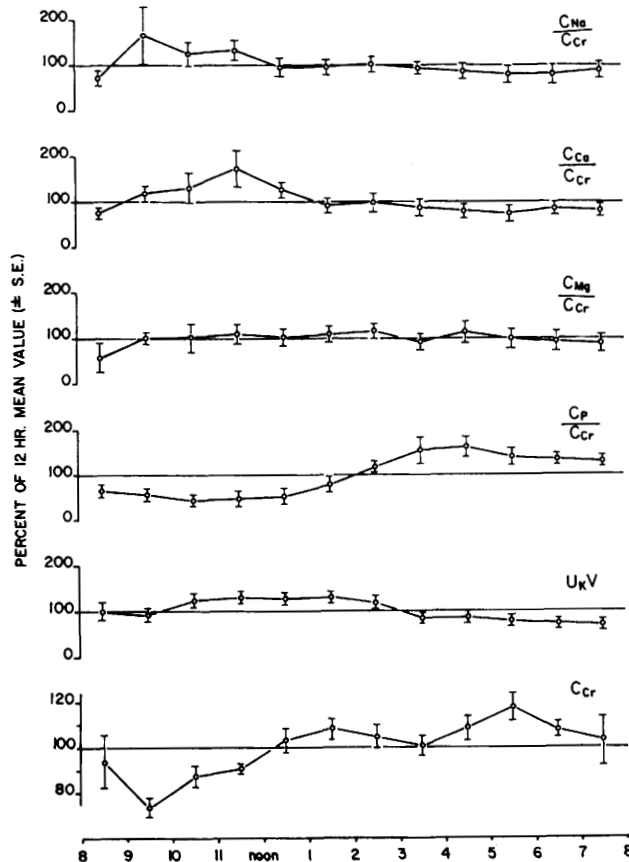


FIG. 2. Pattern of renal function and electrolyte excretion in 6 thyroparathyroidectomized dogs. Data calculated and expressed in the same manner as Fig. 1.

and phosphorus is closely related in the proximal tubule (6-9), and many factors which affect the reabsorption of one are associated with parallel changes in the others. Also, sodium and calcium are reabsorbed throughout the tubule while controversy exists concerning whether phosphorus is reabsorbed solely in the proximal tubule (10) or, to some extent, in the distal tubule as well (11).

If phosphorus reabsorption occurs only in the proximal tubule, one must accept at least two assumptions to account for the present observations. First, the changes in calcium and sodium excretion which were opposite to those of phosphorus should be due to variations in the distal transport of sodium and calcium ions; and second, the increase in the fractional excretion of phosphorus of 10-15% in the early afternoon

should be due to a comparable decrease in phosphorus reabsorption in the proximal tubule. A spontaneous variation of such a magnitude in proximal tubular reabsorption which augments only urinary phosphorus excretion would seem somewhat unlikely.

However, phosphorus reabsorption may occur in the distal nephron (11), where the reabsorption of calcium can be dissociated from that of sodium (12). Thus, it is possible that independent factors, both hormonal and nonhormonal, might independently affect transport of calcium, sodium, and phosphorus in the more distal areas of the nephron to account for the different patterns of their excretions. Although the latter possibility is more attractive, the present observations do not allow us to localize the site of the nephron involved.

The tubular reabsorption of phosphorus

is affected by variations of parathyroid gland activity (13, 14). In the present study, there was a similar pattern of phosphorus excretion in both normal and thyroparathyroidectomized animals although the amplitude of change was greater in the dogs with intact parathyroid glands. These observations suggest that spontaneous variations in the endogenous level of parathyroid hormone are not responsible for the existence of the spontaneous alterations in phosphorus excretion but may contribute to the magnitude of the changes. Unfortunately, there are no available data on diurnal variations in the blood level of parathyroid hormone in the dog, but Jubiz and associates (15) have reported stable levels of blood parathyroid hormone in man during the day.

Finally, the role of changes in glomerular filtration rate should be considered as a possible mechanism underlying the variations in electrolyte excretion. It seems unlikely that a change in glomerular filtration rate with consequent alteration in filtered loads could account for increased sodium and calcium excretion and decreased phosphorus excretion (16). It is possible that the reduced excretion of all substances observed late in the afternoon (5–9 PM) might be due in part to the reduced glomerular filtration rate.

Summary. The daytime pattern of renal excretion of electrolytes in normal and thyroparathyroidectomized dogs is similar. Marked changes in the excretion of phosphorus were observed with dissociation from the patterns of excretion of sodium and calcium. The fractional excretion of phosphorus varied from 0.01 to 0.35 independent of parathyroid hormone or changes in serum

phosphorus.

Technical assistance was provided by John Steppe, Ernest Tallos, and Sandy Howard. Mrs. Catherine Hoyt provided valuable secretarial assistance and Nicholas Bolotine prepared the illustrations.

1. Mills, J. N., and Stanbury, S. W., *J. Physiol.* **115**, 18 (1951).
2. Heaton, F. W., and Hodgkinson, A., *Clin. Chim. Acta* **8**, 246 (1963).
3. Wesson, L. G., *Medicine* **43**, 547 (1964).
4. Massry, S. G., Coburn, J. W., and Kleeman, C. R., *J. Clin. Invest.* **48**, 1237 (1969).
5. Massry, S. G., Ahumada, J. J., Coburn, J. W., and Kleeman, C. R., *Amer. J. Physiol.* **219**, 881 (1970).
6. Walser, M., *Amer. J. Physiol.* **200**, 1099 (1961).
7. Massry, S. G., Coburn, J. W., Chapman, L. W., and Kleeman, C. R., *Amer. J. Physiol.* **213**, 1218 (1967).
8. Staum, B. B., Hamburger, R. J., and Goldberg, M., *J. Clin. Invest.* **51**, 2271 (1972).
9. Rastiger, A., Agus, Z., Connor, T. B., and Goldberg, M., *Kidney Internat.* **2**, 279 (1972).
10. Strickler, J. C., Thompson, D. D., Klose, R. M., and Giebisch, G., *J. Clin. Invest.* **43**, 1596 (1964).
11. Amiel, C., Kuntzinger, H., and Richet, G., *Pflügers Arch.* **317**, 93 (1970).
12. Massry, S. G., Coburn, J. W., Chapman, L. W., and Kleeman, C. R., *Amer. J. Physiol.* **214**, 1403 (1968).
13. Foulks, J. G., and Perry, F. A., *Amer. J. Physiol.* **196**, 567 (1959).
14. Hiatt, H. H., and Thompson, D. D., *J. Clin. Invest.* **36**, 557 (1957).
15. Jubiz, W., Canterbury, J. M., Reiss, E., and Tyler, F. H., *J. Clin. Invest.* **51**, 2040 (1972).
16. Massry, S. G., and Kleeman, C. R., *J. Lab. Clin. Med.* **80**, 654 (1972).

Received Sept. 4, 1973. P.S.E.B.M., 1974, Vol. 145.