proteolytic activator or plasmin activity of normal or traumatized rats.

The author wishes to acknowledge the technical assistance rendered by Mr. Alfred Seaton.

1. Thomas, W. A., O'Neal, R. M., Lee, K. T., Am. Med. Assn. Arch. Path., 1956, v61, 380.
2. Nath, M. C., Saikia, A., J. Nutrition, 1959, v69, 403.
3. Duncan, G. G., Waldron, J. M., Trans. Assn. Am. Phys., 1949, v62, 179.
4. Fullerton, H. W., Davie, W. J. A., Anastasopoulos, G., Brit. Med. J., 1953, v2, 250.
5. Keys, A., Buzina, R., Grande, F., Anderson, J. T., Circulation, 1957, v15, 274.
6. Scott, R. F., Thomas, W. A., Proc. Soc. Exp. Biol. and Med., 1957, v96, 24.
7. Billimoria, J. D., Drysdale, J., Jones, D. C. C., MacLagan, N. F., Lancet, 1959, v2, 471.
8. Gillman, T., Naidoo, S. S., Hathorn, M., ibid., 1957, v2, 696.
9. Merskey, C., Gordon, H., Lackner, H., British Med. J., 1960, v2, 219.
10. Duguid, J. B., Lancet, 1954, v1, 891.
11. Woodman, A. G., Food Analysis, McGraw-Hill, New York, 1941, p199.
12. Jamieson, G. S., Baughman, W. F., Cotton Oil Press, 1924, v7,(12):34.
13. O'Neal, R. M., Still, W. J. S., Hartroft, W. S., J. Path. and Bact., 1961, v82, 183.
14. Fillios, L. C., Andrus, S. B., Mann, G. V., Stare, F. J., J. Exp. Med., 1956, v104, 539.
15. Beard, E. L., Hampton, J. K., Am. J. Physiol., 1963, v204, 405.
16. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., J. Biol. Chem., 1952, v195, 357.
17. McLaughlin, J., Gray, I., Am. J. Physiol., 1959, v196, 893.
18. Grieg, H. B. W., Runde, I. A., Lancet, 1957, v2, 461.

Received August 24, 1966. P.S.E.B.M., 1966, v123.

Determination of Total Cation-Forming Mineral Elements in Feces And Urine and Its Relation to Renal "Net Acid" Excretion.* (31577)

MERRILL N. CAMIEN, LEO M. SMITH,† THOMAS J. REILLY, AND DANIEL H. SIMMONS
Cedars-Sinai Medical Research Institute, and Departments of Medicine and Physiology, University of California Medical Center, Los Angeles

The significance of renal "net acid" excretion, calculated as the sum of urinary titratable acid (TA) and ammonium minus urinary bicarbonate ("net acid" = TA + NH_4^+ − HCO_3^-), in relation to hydrogen ion regulation in the mammalian organism is widely recognized, and a detailed discussion of this subject has been presented by Elkinton(1). Questions concerning the ultimate sources of urinary "acid" have long been of considerable interest. Early investigations reviewed by Shohl(2) tended to emphasize dietary ash as a source of this "acid"; however, a relatively recent study reported by Hunt(3) indicated that it was "impossible to demonstrate any relation between the urinary output of acid and the acidity or alkalinity of the ash of the diet, apart from its sulphur content." This observation has apparently considerably influenced current concepts of acid production as discussed by Relman *et al*(4-6) in their studies concerning "net balance of acid." On the other hand, the present authors(7) have indicated that urinary mineral composition and renal "net acid" excretion are markedly interdependent and that the extent to which cation-forming‡ elements are excreted in the feces can affect the extent of renal "net acid" excretion. The latter observation suggests, since cation-forming elements constitute a major component of both dietary and fecal ash, that the conclu-

---

* Supported by USPHS Grants AM-08523 from Nat. Inst. of Arthritis and Metab. Dis. and HE-08075 from Nat. Heart Inst.

† Supported by University of Southern California Student Research Program, USPHS Grant FR05356-04-5.

‡ Replaces the anachronistic and partly inaccurate term, "base-forming," in reference to elements such as Na, K, Mg and Ca, which exist in biological fluids solely as either free or bound cations.

sion of Hunt(3), as quoted above, might have been appreciably different if the study(3) had taken fecal mineral excretion into account.

The present report describes a relatively simple titrimetric procedure for determining total cation-forming mineral elements in urine and feces and introduces experimental evidence concerning the relationship between urinary and fecal excretion of cation-forming mineral elements and renal "net acid" excretion.

Methods. Six female Wistar rats weighing 155-175 g were maintained in separate metabolism cages on a constant daily neutral-ash ration consisting of 10.0 g of Purina Laboratory Chow Meal mixed with 20.0 ml of 0.175 *N* hydrochloric acid.§ Water was provided *ad lib*, and urine and feces were collected separately and quantitatively as described previously(8). Each 24-hour urine specimen was mixed, during collection, with a mineral-free preservative and diluted to 100 ml as described(8) before sampling for analysis. Renal "net acid" was determined by the procedure of Jørgensen(9), total cation-forming mineral elements were determined as described further on, and individual elements were determined by flame emission spectrophotometry(10) with a Zeiss Model PMQ2 spectrophotometer equipped with double monochrometer MM12 and standard flame accessory. Each 24-hour feces specimen was dried and pulverized as described(8). Three of the six rats, serving as control animals, received the unaltered daily ration for the entire 30-day experimental period. After the first 5 days, the daily diet of each of the remaining (experimental) 3 rats was altered by adding to it the mineral mixture obtained by ashing the preceding day's feces in a muffle furnace for 4 hours at 480°. The altered regimen of the experimental animals was continued for 15 days, after which these animals were returned to the same diet as that received by the controls.

Determination of total cation-forming mineral elements. A 5.00 ml sample of each diluted urine specimen was placed in an accurately tared 20 ml platinum crucible and dried at 80° in an oven. The crucibles, containing the dried samples, and with platinum crucible covers in place, were ignited for 4 hours at 480° in a muffle furnace. Five ml of 0.03 *N* nitric acid were employed to dissolve the cooled ash in each crucible, and 1 ml of water, employed to rinse the corresponding crucible cover, was added to each solution. The ash solutions in the crucibles were heated 20 minutes in an autoclave at 120° and cooled. Water was then added to bring the weight of solution in each crucible to 11.00 g. A 5.00 ml sample of each solution was passed through a separate 10 × 100 mm column of Dowex 50W-X4 (100-200 mesh) resin prepared in the hydrogen form as described previously(8). The solutions emerging from the columns were collected in separate 100 ml beakers, and each sample was rinsed into its beaker with 4 consecutive 5 ml portions of water added to the corresponding column. A second 5.00 ml sample of each ash solution was placed directly (without column treatment) into a 100 ml beaker and diluted with 20 ml of water. All solutions were finally heated for 20 minutes at 120° in an autoclave, then cooled and titrated with standard 0.4 *N* sodium hydroxide solution, delivered from a micro-syringe buret, to the endpoint of pH 4.5 as indicated by a pH meter. Dilutions were calculated on the basis of 11.03 ml for the volume of each ash solution after bringing its weight to 11.00 g, and total cation-forming mineral elements were calculated on the basis that the latter are removed from solution by the column treatment and replaced with a stoichiometrically equivalent amount of titratable hydrogen ion, which is measured by the difference in titration between the treated and untreated sample solutions.

A 300 mg sample of each dried feces sample to be assayed for total cation-forming mineral was accurately weighed into a 20 ml platinum crucible, covered with a little 12 *N* hydrochloric acid, and dried at 80° on a thermostatted hot-plate in a fume hood. This pre-

---

§ Unintentionally slightly more than needed to compensate exactly for the alkaline-ash composition of Purina Laboratory Chow Meal. Mineral composition including added acid (meq per daily ration): Na 1.43, K 2.18, Ca 6.10, Mg 1.57, Cl 4.90, P 5.18, and S 1.82. P is calculated at 1.8 meq/mmol(4).

TABLE I. Recovery of Total Cation-Forming Mineral Elements from Mixture of Organic and Inorganic Electrolytes and Urea.

Element added to mixture[*]						
Na	K	Ca	Mg	Total added	Total found[†]	Recovered[‡]
μeq						%
0	0	0	0	0	1.6 ± .5 (8)	—
149.5	0	0	0	149.5	150.6 ± .2 (4)	99.7
0	140.8	0	0	140.8	141.4 ± .4 (4)	99.3
0	0	163.3	0	163.3	165.6 ± .2 (5)	100.4
0	0	0	148.7	148.7	150.1 ± .5 (5)	99.9
37.4	35.2	40.8	37.2	150.6	151.8 ± .5 (4)	99.7

[*] Amounts per assay sample—added as precisely standardized solutions prepared from analytical reagent grade sodium and potassium hydroxides and calcium and magnesium carbonates. Other components of mixture, in μmol per assay sample, were: citric acid 200, hydrochloric acid 360, sulfuric acid 60, phosphoric acid 60, ammonia 120, arginine 20, histidine 20, lysine 20 and urea 1000.

[†] Mean ± standard error. Number of trials given in parentheses.

[‡] Amount found in basal mixture (1.6 μeq) was assumed to derive from trace contamination with cation-forming elements and was subtracted from other total found values in calculating recovery. Less than 0.03% (by weight) contamination could account for the value found for basal mixture.

liminary acid treatment facilitated subsequent production of a clean, white ash. Ignition and subsequent assay procedure were the same as described for urine except that each ash was dissolved in 5 ml of 0.5 *N* hydrochloric acid, and the ash solution (instead of being diluted in its crucible) was quantitatively transferred to a 100 ml volumetric flask and diluted with water to volume.

Accuracy of the procedure was checked by applying it to a complex mixture of organic and inorganic substances containing known levels of sodium, potassium, calcium and magnesium both singly and in combination. An additional check on accuracy was made by applying the described procedure to urine and feces samples of known mineral composition as established by independent analyses.

Results and discussion. The data of Table I indicate that the described method for determining total cation-forming mineral elements is accurate within tolerance limits of approximately 0.5%. The data of Table II suggest that this method yields values slightly greater than the sum of the values for sodium, potassium, calcium and magnesium in urine and feces samples. The discrepancy is largely accounted for by the cation-forming mineral elements, iron, manganese, zinc and copper, which were present at appreciable levels in the diet[||] but omitted from the urine and feces analyses (Table II). An entirely different method for determining total cation-forming mineral elements in the urine was described by Fiske(12), who obtained excellent results with a mixture of inorganic salts of sodium, potassium, calcium and magnesium (further tests of reliability were not reported); however, the described procedure (12) is relatively cumbersome and was not tested by the present authors.

Mean daily renal "net acid" excretions over each of six 5-day periods are shown for the 3 experimental and 3 control rats in upper part of Fig. 1. Control excretions of renal "net acid" (shaded bars, upper part of Fig. 1) averaged 3.6 meq per day over the total 30-day period despite a constant daily intake of neutral-ash diet.[¶] This result is in accord with the data of Hunt(3), who reported significantly positive renal "net acid" excretions for human subjects on a milk diet and on vegetarian diets of presumably alkaline-ash

---

[¶] Actually slightly acid-ash since daily ration contained a surplus of 0.6 meq of "acid-forming over base-forming" mineral elements. The magnitude of this surplus, however, is only 17% of the mean daily control excretion (3.6 meq) of renal "net acid."

[||] Total meq of Fe, Mn, Zn and Cu (from average of values given in Purina Laboratory Manual) was 1.3% of the total meq of Na, K, Ca and Mg (from authors' analytical values), and values for total cation-forming mineral elements determined titrimetrically in both urine and feces averaged 1.8% more than values calculated from Na, K, Ca and Mg contents.

TABLE II. Cation-Forming Elements in Urine and Feces.

24-hr urine values*							24-hr feces values*						
Na	K	Ca	Mg	Total A†	Total B†	$\frac{100\text{ B}}{\text{A}}$	Na	K	Ca	Mg	Total A†	Total B†	$\frac{100\text{ B}}{\text{A}}$
.55	1.27	.23	.30	2.35	2.37	100.9	.07	.32	5.26	.82	6.47	6.47	100.0
1.18	1.37	.12	.24	2.91	2.95	101.4	.09	.31	4.78	.75	5.93	6.07	102.4
1.10	1.33	.39	.31	3.13	3.18	101.6	.08	.33	4.98	.76	6.15	5.94	96.6
.91	1.38	.08	.20	2.57	2.68	104.3	.13	.37	4.96	.83	6.29	6.23	99.0
.88	1.35	.13	.25	2.61	2.70	103.4	.07	.32	4.77	.76	5.92	6.27	105.9
1.08	1.44	.09	.23	2.84	2.95	103.9	.08	.35	4.96	.85	6.24	6.34	101.6
.85	1.26	.30	.26	2.67	2.64	98.9	.07	.37	4.74	.82	6.00	6.33	105.5
.91	1.42	.30	.31	2.94	2.90	98.6	.03	.32	4.66	.76	5.77	6.06	106.8
.85	1.29	.29	.28	2.71	2.71	100.0	.08	.37	4.57	.69	5.71	6.04	105.8
1.07	1.34	.35	.42	3.18	3.10	97.5	.05	.28	4.92	.76	6.01	6.40	106.5
1.02	1.47	.53	.47	3.49	3.35	96.0	.06	.33	4.91	.73	6.03	6.14	101.8
1.03	1.32	.32	.30	2.97	2.94	99.0	.07	.31	4.82	.71	5.91	6.13	103.7
1.25	1.56	.44	.33	3.58	3.61	100.9	.07	.36	5.04	.77	6.24	6.38	102.2
1.08	1.64	.40	.31	3.43	3.51	102.3	.07	.38	4.70	.77	5.92	6.20	104.7
Mean ± standard deviation													
.98	1.39	.28	.30	2.96	2.97	100.6	.07	.34	4.86	.77	6.04	6.21	103.0
±.18	±.11	±.14	±.07	±.37	±.35	±2.4	±.02	±.03	±.18	±.05	±.21	±.16	±3.1

* Values in meq/24 hr for 14 urine and feces samples from rats maintained on 8.0 g of Purina Laboratory Chow Meal per day, in some instances with a daily supplement of L-methionine (provides no additional cation-forming elements), as described previously(8). Values for Ca determined by chelometric titration(11) and for other elements by flame photometry(10).

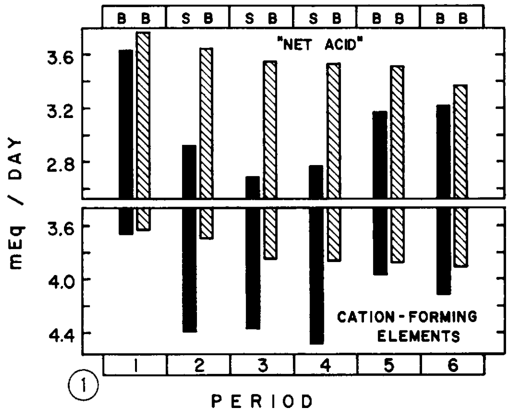
† Total A indicates sum of Na, K, Ca and Mg values. Total B is value determined titrimetrically for total cation-forming elements.

composition. However, human infants on milk-formula diets excrete appreciably more total amounts of cation-forming mineral elements in the feces than in the urine according to data reported by Shohl(2), and a similar relationship between urinary and fecal excretion of cation-forming mineral elements is evident from the data of Table II for rats maintained on a basal ration of Purina Laboratory Chow. Hence, the relatively high renal "net acid" excretion values for both Hunt's subjects(3) and the present control animals (Fig. 1) might largely be accounted for by relatively high fecal excretions of cation-forming mineral elements, which would leave relatively less cation-forming mineral elements for excretion in the urine. The latter would tend to be associated with relatively high renal "net acid" excretion values because of the inverse relationship between urinary levels of total cation-forming mineral elements and renal "net acid" values, which is evident from the following equations.

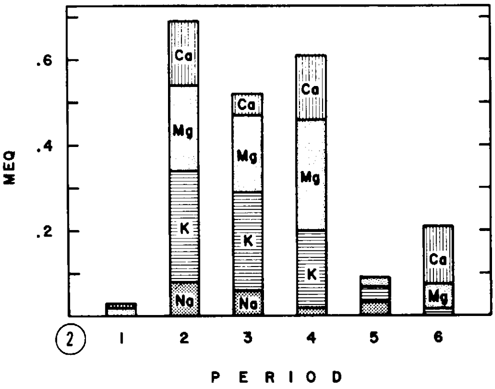
1. $\text{meq }(\text{TA} + \text{NH}_4^+ + \text{Na}^+ + \text{K}^+ + \text{Ca}^{++} + \text{Mg}^{++} + \text{OB}\cdot\text{H}^+) = \text{meq }(\text{SO}_4^= + \text{HPO}_4^= + \text{H}_2\text{PO}_4^- + \text{Cl}^- + \text{OA}^- + \text{HCO}_3^-)$

2. $\text{meq }(\text{TA} + \text{NH}_4^+ - \text{HCO}_3^-) = \text{meq }(\text{SO}_4^= + \text{HPO}_4^= + \text{H}_2\text{PO}_4^- + \text{Cl}^-) - \text{meq }(\text{Na}^+ + \text{K}^+ + \text{Ca}^{++} + \text{Mg}^{++}) + \text{meq }(\text{OA}^- - \text{OB}\cdot\text{H}^+)$

Equation 1 represents the relationship which must hold for parity of opposing ionic charges in urine which has been titrated with sodium hydroxide to blood pH. The TA value equals cations added with the titrant, so it is included with the urinary cations on the left side of Equation 1. The designations, $\text{OB}\cdot\text{H}^+$ and OA^-, represent cations derived by protonation of urinary organic bases and anions derived by deprotonation of urinary organic acids, respectively. Inorganic ions other than those included in Equation 1 are normally present in only trace amounts in urine and are regarded as negligible. Equation 2 is simply an algebraic rearrangement of Equation 1, illustrating that the renal "net acid" value, which is traditionally expressed in terms of TA, NH_4^+ and HCO_3^-, may also be expressed in terms of other urinary ions. Since the cation-forming mineral elements are entirely present as inorganic cations in urine, and since the sum of the latter ions appears as a negative component of the renal



①



②

FIG. 1. Mean daily excretions of renal "net acid" (upper) and total urinary cation-forming mineral elements (lower) by rats of experimental group (solid black bars) and control group (shaded bars) over 6 consecutive 5-day periods. Notation B, at top of Figure, designates data corresponding to intake of basal ration alone. Notation S designates data corresponding to intake of rations supplemented with fecal minerals over periods 2-4 (experimental group only) as described in text.

FIG. 2. Mean daily values for individual cation-forming mineral elements excreted in urine by experimental rats minus corresponding values for control animals. Values for individual elements are plotted cumulatively to show total increments of cation-forming mineral elements corresponding to the differences between experimental and control values in Fig. 1.

"net acid" value as expressed in Equation 2, it is clear that any increase in urinary excretion of total cation-forming mineral elements, when excretion of other urinary ions does not change, must be accompanied by an equivalent decrease in renal "net acid" excretion.

The present data indicate that feeding fecal minerals to the experimental rats (solid black bars, periods 1-4, Fig. 1) effects an appreciable reduction of renal "net acid" excretion together with a corresponding increase in urinary excretion of cation-forming mineral elements by the latter animals relative to excretions of the controls (shaded bars, Fig. 1). A reasonable interpretation of these data is that cation-forming mineral elements originally excreted in the feces were ultimately (after adding the fecal minerals to the diet) absorbed and excreted in the urine, an accomplishment associated with reduced renal "net acid" excretion for the reasons already discussed. This interpretation is supported by the data of Fig. 2, which indicate that the increments of total urinary cation-forming mineral elements associated with the decrements of renal "net acid" excretion under these conditions (Fig. 1) are largely accounted for by increments of urinary calcium and magnesium, elements which predominate in the fecal ash but which normally account for only a relatively small fraction of the total urinary inorganic cations (Table II). Thus, although the factors which determine distributions of cation-forming mineral elements between urine and feces remain largely unknown, the present data indicate that the extent to which these elements are excreted in the feces rather than in the urine can considerably influence the extent of renal "net acid" excretion.

Summary. A relatively simple titrimetric method for determining total cation-forming mineral elements in urine and feces is described and demonstrated to yield dependable values. Addition of fecal minerals to an otherwise constant diet in rats is shown to effect an increased urinary excretion of total cation-forming mineral elements together with a correspondingly decreased renal "net acid" excretion. The theoretical basis for this result and for analogous results of others is discussed. It is concluded that the extent to which cation-forming mineral elements are excreted in the feces rather than in urine can considerably influence the extent of renal "net acid" excretion.

ADDENDUM: While the preceding report was in press, studies by Lennon, Lemann and

Litzow(13,14) appeared, in which collective values for Na^+, K^+, Ca^{++} and Mg^{++} (ions derivable from cation-forming elements) in diet and feces, respectively, are calculated as a negative and positive component, respectively, of "total effective acid production" ("TEAP") in the organism, a value which is shown normally to be balanced by renal "net acid" excretion(13). The value for fecal excretion of the cation-forming mineral elements therefore appears as a positive component of the "TEAP" value, and since the latter is normally balanced by the renal "net acid" value, it appears that the described calculation of "TEAP"(13) takes into account the influence of fecal excretion of cation-forming mineral elements on renal "net acid" excretion. However, the nature of the calculation suggests that the authors(13) are unaware of the relationships shown in Equation 2, which account for this influence. The complete operational definition proposed(13) for "TEAP" may be expressed by the following equation, in which "absorbed" designates values obtained by subtracting fecal from dietary values:

3. meq "TEAP" $=$ meq urinary $(SO_4^{=} + OA^-)$ — meq absorbed $(Na^+ + K^+ + Ca^{++} + Mg^{++} - Cl^-) + 1.8 \times$ mmoles absorbed P

However, if the ratio of $HPO_4^{=}$ to $H_2PO_4^-$ in urine after titration to blood pH is taken as 4 to 1, and if the value of urinary P beyond that represented by orthophosphate ions is taken to be negligible, then the value of meq urinary $(HPO_4^{=} + H_2PO_4^-)$ in Equation 2 equals $1.8 \times$ mmoles urinary P, and Equation 2 may be rearranged to:

4. meq "net acid' $=$ meq urinary $(SO_4^{=} + OA^-)$ — meq urinary $(Na^+ + K^+ + Ca^{++} + Mg^{++} - Cl^-) + 1.8 \times$ mmoles urinary P — meq urinary $OB \cdot H^+$

When the definitions of "TEAP" and "net acid" are expressed in terms of organic and inorganic ions and phosphorus as in Equations 3 and 4 and are inspected simultaneously, it becomes obvious that regardless of whether or not the "TEAP" value is quantitatively representative of endogenous acid production (the present authors suggest that it is not), it must nevertheless be balanced by the renal "net acid" excretion value whenever net absorption of Na^+, K^+, Ca^{++}, Mg^{++}, Cl^- and P is balanced by urinary excretion of these minerals, providing the value of urinary $OB \cdot H^+$ (as seems likely) remains negligible.

---

1. Elkinton, J. R., Ann. Intern. Med., 1962, v57, 660.
2. Shohl, A. T., Physiol. Rev., 1923, v3, 509.
3. Hunt, J. N., Clin. Sci., 1956, v15, 119.
4. Relman, A. S., Lennon, E. J., Lemann, J., Jr., J. Clin. Invest., 1961, v40, 1621.
5. Goodman, A. D., Lemann, J., Jr., Lennon, E. J., Relman, A. S., ibid., 1965, v44, 495.
6. Lemann, J., Jr., Lennon, E. J., Goodman, A. D., Litzow, J. R., Relman, A. S., ibid., 1965, v44, 507.
7. Camien, M. N., Smith, L. M., Simmons, D. H., Fed. Proc., 1966, v25, 203.
8. Camien, M. N., Anal. Biochem., 1966, v15, 127.
9. Jørgensen, K., J. Clin. and Lab. Invest., 1957, v9, 287.
10. Dean, J. A., Flame Photometry, McGraw-Hill Series in Advanced Chemistry, McGraw-Hill Co., New York, 1960.
11. Diehl, H., Ellingboe, J. L., Anal. Chem., 1956, v28, 882.
12. Fiske, C. H., J. Biol. Chem., 1922, v51, 55.
13. Lennon, E. J., Lemann, J., Jr., Litzow, J. R., J. Clin. Invest., 1966, v45, 1601.
14. Lemann, J., Jr., Litzow, J. R., Lennon, E. J., ibid., 1966, v45, 1608.

Received August 24, 1966. P.S.E.B.M., 1966, v123.