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Relationship of Renal "Net Acid" Excretion to Titratable Ash-Acidity (Ash-TA) in Diet and Feces.* (32364)

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Recent studies concerning quantitative relationships between endogenous acid production and renal "net acid" excretion have been referred to elsewhere(1), and earlier work in this area has been reviewed by Elkinton(2). It is generally accepted that renal "net acid" excretion can be quantitatively evaluated in terms of urinary titratable acidity (TA), ammonium and bicarbonate as represented in Equation 1, Table I. However, evaluations of endogenous acid production have been unsatisfactory from both conceptual and methodological standpoints. Goodman *et al*(3) equated endogenous acid production solely to the sum of values for urinary sulfate and organic anions, although the earlier procedure of Relman *et al*(4) included also the calculated value for acid phosphorus from the diet as a component of the endogenous acid production value. More recently, Lennon *et al* (5) modified the method of Goodman *et al*(3) by including the value of absorbed (dietary minus fecal) chloride and phosphorus minus absorbed sodium, potassium, calcium and magnesium as an additional component of the endogenous acid production value. All of these procedures yield values which appear to represent endogenous acid production under the described conditions(3-5) since the values agree satisfactorily with renal "net acid" excretion values for normal, unstressed human subjects. However, it has subsequently been pointed out(1) that the values for urinary sulfate and organic anions must inevitably constitute components of the corresponding renal "net acid" values regardless of endogenous acid production (see Equations 1-3, Table I). On this basis, it was shown(1) that an endogenous acid production value calculated as described by Lennon *et al*(5) must unavoidably tend to equal the corresponding renal "net acid" value, regardless of actual endogenous acid production, providing balance is maintained between mineral intake and excretion. The same must also apply to endogenous acid production values calculated as described by Relman and his associates(3,4).

Although there is no evidence that either mineral intake or excretion can provide an adequate basis for estimating true endogenous acid production, the relationships shown in Equation 3, Table I, suggested that the renal "net acid" excretion value might be determined largely by mineral composition of the urine, and data presented earlier(1) verified that the extent to which cation-forming mineral elements are absorbed from the gut can considerably influence the extent of renal "net acid" excretion. The present report is concerned with both cation-forming and anion-forming mineral elements in terms of a parameter which we have named titratable

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TABLE I. Equations Relating Renal "Net Acid" Value to Urinary Ash-TA Value.

1. meq "Net acid" = meq ($TA + NH_4^+ - HCO_3^-$)*
2. meq ($TA + Na^+ + K^+ + Ca^{++} + Mg^{++} + NH_4^+ + OB \cdot H^+$) = meq ($SO_4^= + HPO_4^= + H_2PO_4^- + Cl^- + HCO_3^- + OA^-$)†
3. meq "Net acid" = meq ($SO_4^= + HPO_4^= + H_2PO_4^- + Cl^-$) − meq ($Na^+ + K^+ + Ca^{++} + Mg^{++}$) + meq ($OA^- - OB \cdot H^+$)‡
4. meq Ash-TA = meq (S + P + Cl) − meq (Na + K + Ca + Mg)§
5. meq Ash-TA = meq ($SO_4^= + HPO_4^= + H_2PO_4^- + Cl^-$) − meq ($Na^+ + K^+ + Ca^{++} + Mg^{++}$) + meq (OS + OP)‖
6. meq "Net acid" = meq ash-TA + meq ($OA^- - OS - OP - OB \cdot H^+$)
7. meq Ash-TA = meq ($TA + NH_4^+ + OS + OP + OB \cdot H^+ - HCO_3^- - OA^-$)

* TA designates titratable acidity of the urine measured by titration with standard base to blood pH.

† Symbols other than TA refer to ions in urine which has been titrated to blood pH. OA^- and $OB \cdot H^+$ designate ions derived by deprotonation of organic acids and by protonation of organic bases, respectively. The TA value equals the value of cation introduced with the titrant. This equation must hold, therefore, if positive ionic charges balance negative ionic charges in the titrated urine (1).

‡ This equation is a rearrangement of Equation 2.

§ Symbols refer to urinary elements with Na, K and Cl evaluated at 1 meq/mmol, Ca, Mg and S at 2 meq/mmol, and P at 1.8 meq/mmol. These evaluations correspond to electrical charge values for ionic derivatives of these elements in extracellular fluid at pH 7.40. The value of 1.8 meq/mmol for P corresponds to the mean charge value for $HPO_4^=$ and $H_2PO_4^-$ in extracellular fluid(4).

‖ Symbols refer to urinary ions except OS for urinary organic sulfur calculated at 2 meq/mmol of sulfur and OP for urinary organic phosphorus calculated at 1.8 meq/mmol (on basis given in footnote §).

ash-acidity (ash-TA), calculated as indicated in Equation 4, Table I. This name is essentially descriptive since the ash-TA value calculated for a given sample of any biological material (urine, feces, foodstuffs) is theoretically equal to the titratable acidity value of the ash (mineral residue) which would be produced by combustion of the sample(6). The experimental data indicate a close correlation between urinary ash-TA and renal "net acid" excretion values and suggest that renal "net acid" excretion relates more closely to net absorption of dietary ash-TA than to endogenous acid production.

Methods. General quantitative procedures relating to use of metabolism cages, feeding, and collection of excreta were essentially the same as described previously(7). The experiments employed 31 male and female 100-200 g Wistar rats and 23 dietary regimens consisting of a) Purina Laboratory Chow Meal; b) 66.5% sucrose, 4.6% corn oil, 23.1% purified casein, 0.4% purified vitamins, 5.4% purified mineral salts;† c) wheat germ meal; d) 90% egg yolk solids, 10% cellulose; e) 90% milk solids, 10% cellulose; f) one or more modifications of each of the preceding diets made by adding various mineral acids, mineral salts, L-methionine and acetazolamide either singly or in combinations. Ash-TA values were determined for diet, urine and feces essentially by titrating the ash produced by combustion of these products(6). Renal "net acid" was measured by the procedure of Jørgensen(8), and urinary "organic acids" were determined essentially as described by Van Slyke and Palmer(9).

Results. Close correlations between urinary ash-TA and renal "net acid" excretion values were consistently found. Such a correlation is readily evident in Fig. 1, which shows the daily excretions of both renal "net acid" and urinary ash-TA for a representative animal maintained on Purina Laboratory Chow Meal with and without L-methionine supplementation. The correlation for all urines assayed for both renal "net acid" and ash-TA (201 urine specimens from 31 rats on 23 different diets) is shown in Fig. 2.

Renal "net acid" values were consistently somewhat higher than the corresponding urinary ash-TA values. The mean (±S.D.) difference between the two values was 0.36 ± 0.13 meq/day in the 23 urine specimens represented in Fig. 1 and 0.42 ± 0.22 meq/day in the 201 urine specimens represented in Fig. 2. This difference was thus seen to be small and essentially constant relative to

† Individual salts, in g per 100 g of diet, were: $CaHPO_4 \cdot 2H_2O$ 1.78, $NaHCO_3$ 1.68, $MgSO_4 \cdot 7H_2O$ 0.94, $KHCO_3$ 0.45, $CaCO_3$ 0.35, $FeSO_4 \cdot 7H_2O$ 0.13, $MnCl_2 \cdot 4H_2O$ 0.025, $CuSO_4 \cdot 5H_2O$ 0.004, $ZnSO_4 \cdot 7H_2O$ 0.0017, and NaI 0.0003. Carbonates and bicarbonates were replaced by equivalent amounts of the corresponding chlorides in modified diets.

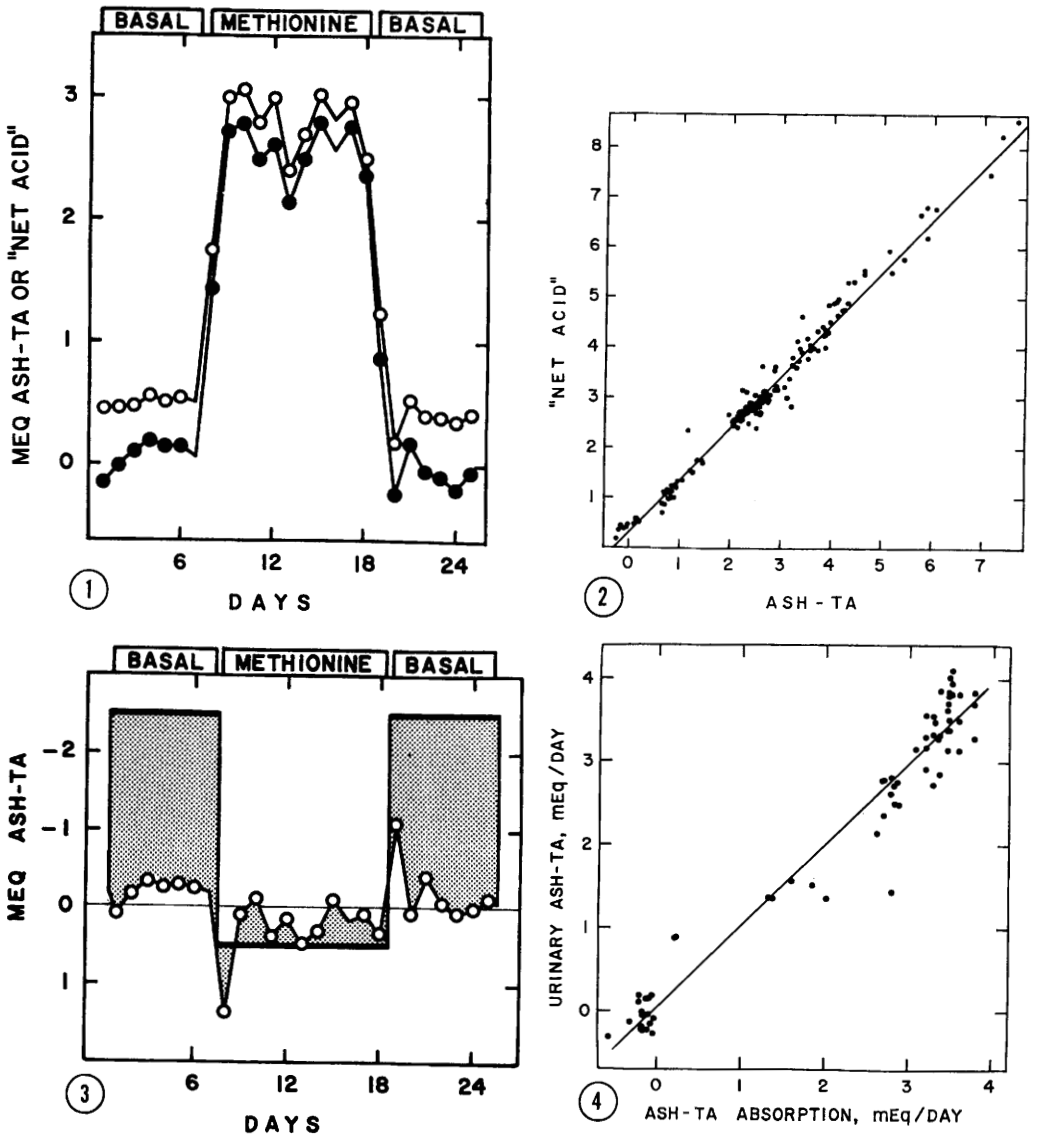

FIG. 1. Daily renal "net acid" excretion (open circles) and urinary ash-TA excretion (solid circles) by a male Wistar rat maintained on 8.00 g of Purina Laboratory Chow Meal per day with and without daily supplement of 1.50 mmole of L-methionine. The animal weighed 112.9, 113.3, 113.3 and 112.4 g on days 0, 7, 16 and 26 (in morning before feeding), respectively.

FIG. 2. Correlation between renal "net acid" and urinary ash-TA. $Y = 0.324 + 1.042\ X$; $r = 0.98$. Values (meq/24 hr) for urines from 31 rats on 23 modifications of 5 different basal diets (see *"Methods"*).

FIG. 3. Ash-TA balance values for experiment described in Fig. 1. Intake levels are represented by heavy horizontal lines (based on mean $\pm$ S. D. of mean value of -31.4 ± 0.4 meq% ash-TA found for 5 representative samples of Purina Laboratory Chow Meal, assuming 2 meq of S per mmole of L-methionine). Positive ash-TA excretion values (meq/24 hr total in urine and feces) are plotted upward from the intake levels and negative values downward. Each circle above the zero line thus indicates a negative ash-TA balance value, and each circle below the zero line represents a positive ash-TA balance value.

FIG. 4. Correlation between net absorption of ash-TA and urinary ash-TA excretion. $Y = 0.064 + 0.969\ X$; $r = 0.98$. The not absorption values are calculated as ash-TA in daily ration minus ash-TA lost in feces. All values are in meq/24 hr.

variations in renal "net acid" excretion imposed by changes in diet. Calculations from data shown in Table II suggest that most of the difference value is generally accounted for by the urinary OA^- value.

Dietary ash-TA intake values corresponding to the urinary excretion values shown in Fig. 1 were balanced by urinary plus fecal ash-TA excretion values as shown in Fig. 3. The mean steady state balance values corresponding to the data of Fig. 3 were —0.19, +0.19 and —0.06 meq of ash-TA per day for days 1-6, 9-18 and 20-25, respectively.

Results of balance experiments with 12

TABLE II. Urinary Organic Anions Value as Component of Difference Between Renal "Net Acid" Value and Urinary Ash-TA Value.

Value	Mean (± S.D.) daily excretion*		
	Days 1-6	Days 9-18	Days 20-25
	meq	meq	meq
1. Renal "net acid"†	.50 ± .06	2.82 ± .25	.38 ± .12
2. Urinary ash-TA†	.06 ± .13	2.57 ± .22	—.10 ± .15
3. $(OA^- - OS - OP - OB \cdot H^+)$‡	.44 ± .09	.25 ± .07	.48 ± .07
4. meq "organic acids"†	.60 ± .08	.61 ± .05	.70 ± .13
5. $(OS + OP + OB \cdot H^+)$§	.16 ± .02	.36 ± .07	.22 ± .15

* Values correspond to data shown in Fig. 1. Days 1-6 (n = 6) and 20-25 (n = 6) represent periods of stable excretion on diet of Purina Laboratory Chow alone. Days 9-18 (n = 9) represent period of stable excretion on same diet supplemented with 1.50 mmole of L-methionine per day.

† Experimentally determined values.

‡ Calculated as renal "net acid" value minus urinary ash-TA value (see Equation 6, Table I).

§ Calculated as "organic acids" value (assuming "organic acids" to be completely ionized at blood pH) minus the value of $OA^- - OS - OP - OB \cdot H^+$.

TABLE III. Ash-TA Balance in Rats Maintained on Semi-Synthetic Diets.*

3-Day period	Diet ingested (g/day)	Ash-TA (meq/day)				
		In diet	In urine	In feces	In urine + feces	Balance†
		Diet ash-TA = —16.0 meq %				
1	9.54	—1.53	—.23	—1.42	—1.65	+.12
2	9.41	—1.51	—.22	—1.32	—1.54	+.03
3	8.94	—1.43	—.31	— .86	—1.17	—.26
4	8.94	—1.43	—.28	—1.39	—1.67	+.24
5	9.22	—1.48	—.14	—1.16	—1.30	—.18
		Diet ash-TA = +5.2 meq %				
6	8.16	+.42	+1.35	—1.60	—.25	+.67
7	8.09	+.42	+1.56	—1.18	+.38	+.04
8	7.84	+.41	+1.35	— .97	+.38	+.03
9	8.03	+.42	+1.51	—1.43	+.08	+.34
10	6.88	+.36	+1.36	— .97	+.39	—.03
		Diet ash-TA = +25.8 meq %				
11	6.66	+1.72	+3.48	—1.59	+1.89	—.17
12	7.75	+2.00	+3.28	—1.35	+1.93	+.07
13	8.03	+2.07	+3.16	—1.01	+2.15	—.08
14	7.36	+1.90	+3.18	—1.30	+1.88	+.02
15	6.59	+1.70	+2.82	—1.09	+1.73	—.03

* Data from analyses of pooled specimens from 3 female Wistar rats (mean weight 228 ± 4 g on first day and 238 ± 1 g on last day of total 45 day experimental period). The basal diet contained sucrose 66.5%, purified casein 23.1%, corn oil 4.6%, purified vitamins 0.4% and purified mineral salts 5.4%. Mineral composition (including mineral salts) except for chloride was constant as follows (values in meq % on same basis as in Equation 3, Table I): Na 21.7, K 4.51, Ca 28.65, Mg 7.62, Mn 0.26, Fe 0.96, Cu 0.03, Zn 0.01, P 28.4 and S 18.4. Chloride was adjusted to values (meq %) of 0.85, 22.09 and 42.75 to yield ash-TA values (meq %) of —16.0, +5.2 and +25.8, respectively.

† Mean (± S.D. of mean; n = 15) balance = 0.05 ± 0.06 meq/day.

TABLE IV. Ash-TA Balance in Rats on Semi-Synthetic Diet Supplemented with Mineral Acids.*

3-Day period	Renal "net acid" excretion (meq/day)	Ash-TA excretion (meq/day) In urine	Ash-TA excretion (meq/day) In feces	Ash-TA balance† (meq/day)
	Rats 1-3. Diet plus HCl. Ash-TA intake 2.96 meq/day.			
1	3.40 ± .11	3.16 ± .07	—.65 ± .09	+.45 ± .02
2	4.16 ± .09	3.76 ± .07	"	—.15 ± .05
3	4.13 ± .12	3.53 ± .09	"	+.08 ± .01
	Rats 4-6. Diet plus H_2SO_4. Ash-TA intake 2.96 meq/day.			
1	3.11 ± .05	2.84 ± .06	—.33 ± .05	+.45 ± .09
2	4.00 ± .03	3.66 ± .10	"	—.38 ± .07
3	3.84 ± .08	3.33 ± .01	"	—.04 ± .04
	Rats 7-9. Diet plus H_3PO_4. Ash-TA intake 2.96 meq/day.			
1	4.27 ± .19	3.64 ± .13	—.53 ± .01	—.15 ± .12
2	4.65 ± .13	3.97 ± .09	"	—.49 ± .08
3	4.21 ± .22	3.77 ± .13	"	—.28 ± .13

* Each tabulated value is a mean ± its standard error. The 9 experimental rats were male Wistars of nearly equal weight (means were 190 ± 2 g at beginning and 198 ± 2 g at end of 9-day experimental period). Diet was the same as in Table III except for adjustments of mineral salts to bring basal ash-TA value to —0.54 meq %. Daily ration (completely consumed each day) for each rat was 8.00 g of basal diet mixed with 2.00 ml of water containing 3.00 meq of the indicated acid. Urine collections were pooled over 3-day periods, and fecal collections were pooled over the entire 9-day period.

† Mean (± S.D. of mean; n = 27) balance = —0.07 ± 0.07 meq/day.

additional rats with dietary ash-TA intake varied in different ways are shown in Tables III and IV. The correlation between net absorption of ash-TA (ash-TA in daily ration minus ash-TA lost in feces) and urinary excretion of ash-TA for all balance experiments (all data for the 13 rats, 8 dietary regimens and 65 collection periods described in Fig. 3 and in Tables III and IV) is shown in Fig. 4.

Discussion. Ash-TA values are strictly defined by mineral composition in terms of chemical elements which can be neither created nor destroyed by metabolism. Consquently, if an organism is to maintain its characteristic mineral composition, its net absorption of dietary ash-TA, which can vary widely according to dietary mineral composition, must, in the long run, be balanced by its urinary ash-TA excretion. Evidence that such a balance is generally achieved by rats on diets of widely differing mineral composition is provided by the experimental data (Figs. 3 and 4, Tables III and IV).

This tendency for urinary ash-TA excretion to match net absorption of dietary ash-TA over wide ranges of dietary intake creates a parallel tendency for urinary ash-TA values to correlate with corresponding renal "net acid" values which is readily evident from the experimental data (Fig. 1 and 2). The reason for this is illustrated by equations in Table I. These show that each urinary ash-TA value (markedly influenced by diet composition) constitutes a major component of the corresponding renal "net acid" value, the remaining components of which are relatively little influenced by the diet.

Equation 1, Table I, represents the conventional definition of renal "net acid"(1-6). Equations 2 and 3 follow because of relationships imposed by parity of opposing ionic charges in urine(1). Equation 4 constitutes a formal definition of the parameter which we have named titratable ash-acidity (ash-TA). Equation 5 is an alternative definition of ash-TA in terms of ions derivable from the mineral elements and of organic sulfur and organic phosphorus. The two definitions (Equations 4 and 5) are evidently equivalent if the assumptions are valid, as is generally presumed, that none of the elements represented in Equation 4 exist in urine in appreciable amounts as constituents of inorganic ions other than those represented in Equation 5 and that none of the elements other than sulfur and phosphorus exist in the urine

in appreciable amounts in organic combinations. Since the ions in Equation 5 are represented on the same basis as in Equation 3, the terms in which the ions appear may be eliminated by subtracting Equation 5 from Equation 3 to yield Equation 6, which indicates that the renal "net acid" value is equal to the urinary ash-TA value (markedly influenced by diet composition) plus the value of the quantity, $OA^- - OS - OP - OB \cdot H^+$. The value of the latter quantity equals the difference between the renal "net acid" value and the urinary ash-TA value and is largely accounted for by the urinary OA^- value according to evidence presented in Table II. The Table II data and related data in paragraph 2 of *Results* indicate that the value of $OA^- - OS - OP - OB \cdot H^+$ in urine remains comparatively small and relatively constant over wide ranges of renal "net acid" excretion.

Rearrangement of Equation 6, Table I, yields Equation 7, which reveals a significant analogy between the urinary ash-TA value and the renal "net acid" value (Equation 1, Table I) in that both contain a "true" acid value (TA, which is subject to evaluation by conventional acidimetry) together with other values concerning substances which may be regarded as either "potential" acids or "potential" anti-acids. The "potential" acid value of cations derivable by protonation of urinary bases (NH_4^+ from NH_3, RNH_3^+ from RNH_2, and $OB \cdot H^+$ from OB) has been discussed elsewhere(10), and the "potential" acid value of organic sulfur (OS) and organic phosphorus (OP) is widely recognized(1-6). The "potential" anti-acid value of organic anions (OA^-) and bicarbonate (HCO_3^-)‡ is also generally recognized, and has recently been described as "potential alkali" by Lennon *et al*(5). When renal excretion is considered in terms of substances actually removed from the organism, therefore, (rather than of mechanisms involved) it is evident that the renal "net acid" value and the urinary ash-TA value each constitutes an approximate evaluation of "potential" acidity§ which has been removed from the organism by renal excretion.

Summary. Titratable ash-acidity (ash-TA) has been described as an acid-base parameter which is based solely on mineral composition and may therefore be defined strictly in terms of biologically stable chemical elements. Renal "net acid" values were shown to consist of two components: 1) the urinary ash-TA value, which varies markedly with diet composition and remains generally equal to the value for net absorption of dietary ash-TA, and 2) a relatively small and essentially constant quantity equal to the urinary organic anions value minus the values for urinary organic sulfur, organic phosphorus and organic cations. Most of the second component was accounted for by the organic anions value, so that the renal "net acid" values were generally slightly higher than the corresponding urinary ash-TA values. The renal "net acid" values nevertheless remained closely correlated with the corresponding urinary ash-TA values and hence with the values for net absorption of dietary ash-TA. No basis was found for relating either ash-TA intake or excretion to true endogenous acid production; however, it was shown that both ash-TA values and renal "net acid" values correspond theoretically to approximate evaluations of "potential" acid.

‡ A major part of the deacidification normally expected to follow HCO_3^- administration cannot be realized until an equivalent amount of carbonic acid (as CO_2) has subsequently been eliminated(11). In this sense, the anti-acid potency of HCO_3^- is largely "potential" rather than "immediate."

§ Substances represented by OS, OP and $OB \cdot H^+$ have "potential" acidity and substances represented by OA^- have "potential" anti-acidity only if they are metabolizable(3-5,10). Since metabolizability is characteristic probably of many, but not necessarily all urinary substances represented by these symbols, neither the renal "net acid" value nor the urinary ash-TA value can be regarded as a theortically *exact* representation of "potential" acid excretion.

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Determination of Titratable Ash-Acidity (Ash-TA).* (32365)

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Titratable ash-acidity (ash-TA) is an acid-base parameter based solely on mineral composition and defined strictly in terms of biologically stable chemical elements(1). Its significance relative to renal "net acid" excretion has been examined and described elsewhere(1). By definition(1), its value may be calculated from separately determined values for Na, K, Ca, Mg, S, P and Cl; however, evaluation of ash-TA in this manner, unless the mineral values are already at hand, involves numerous procedural operations in the required analyses. The present report describes a relatively simple and essentially direct titrimetric procedure for determining ash-TA.

Methods. Urine, feces and diet samples were the same as described previously(2). Determinations of sodium, potassium, calcium, magnesium(3) and sulfur(2) were as described. Chloride was measured amperometrically(4). Phosphorus and ash-TA were determined as follows.

Principle of ash-TA determination. Combustion of a sample of biological material in presence of a measured excess of sodium carbonate removes organic and nitrogenous components and leaves mineral components as residual ash (incidental sulfur losses are determined and corrected for, when necessary, as described further on). Dissolving the ash in measured excess of dilute hydrochloric acid with controlled heating decomposes carbonates and hydrolyses meta- and pyrophosphates, leaving cation-forming elements as Na^+, K^+, Ca^{++} and Mg^{++} and anion-forming elements as Cl^-, $SO_4^=$, $H_2PO_4^-$, HSO_4^- and H_3PO_4. Titration of the solution with sodium hydroxide to pH 4.5 (first endpoint) introduces additional Na^+ and converts HSO_4^- and H_3PO_4 to $SO_4^=$ and $H_2PO_4^-$, respectively. Hence, when the first endpoint of the titration is reached, each mineral element in the titration mixture is represented by only one ionic species, and the meq of cation-forming elements (including Na^+ from titrant) necessarily equal the meq of anion-forming elements at this point on the basis (from ionic charge numbers) of 1 meq/mmole for Na, K, Cl and P and 2 meq/mmole for Ca, Mg and S. In reaching this point, the surplus of anion-forming over cation-forming elements initially present because of the Cl introduced as dilute hydrochloric acid in dissolving the ash must be precisely cancelled by the amount of cation-forming element added as Na^+ from the titrant during the course of the titration. Therefore, the meq of titrant required to bring the ash solution to pH 4.5 is equal to the initial surplus of anion-forming over cation-forming mineral elements in the solution on the stipulated equivalence basis. Addition of excess neutral calcium chloride solution and continuing the titration to pH 8.5 (second endpoint) converts $H_2PO_4^-$ to $Ca_3(PO_4)_2$ at the expense of 2 meq of OH^-

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