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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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(from titrant) per mmole of $H_2PO_4^-$ (the excess Ca^{++} added as calcium chloride solution insures that all of the $H_2PO_4^-$ will be titrated to $Ca_3(PO_4)_2$, rather than to $HPO_4^=$, which would require only 1 meq of titrant per mmole of $H_2PO_4^-$). Ions other than $H_2PO_4^-$ are left unchanged during titration from the first to the second endpoint. Hence, total meq of titrant to endpoint 2 minus the meq of titrant to endpoint 1 equals the meq of P in initial solution calculated at 2 meq/mmole. Also, the meq of Na^+ added with total titrant to endpoint 2 corresponds to the initial surplus of anion-forming over cation-forming elements in the solution calculated as before except with P at 3 meq/mmole. Hence, the meq of titrant to endpoint 1, plus four-tenths the additional meq of titrant to endpoint 2, equal the surplus of anion-forming over cation-forming mineral elements with P at 1.8 meq/mmole. The surplus, on this basis (and corrected for the extraneous sodium carbonate and hydrochloric acid added in measured amounts in preparing the ash and dissolving it), equals the meq of ash-TA in the initial sample according to the given definition(1).

Procedure for urinary phosphorus and ash-TA. The urine samples were diluted and contained preservative as described(2). A 5.00 ml sample of diluted urine together with a precisely measured volume of *N* sodium carbonate solution (either 150 or 300 μl according to anticipated sample level of ash-TA) was dried at 80° in a 20 ml platinum crucible (with cover) and ignited for 4 hours at 480° in a muffle furnace. The cooled ash was dissolved in 5.00 ml of 0.06 *N* hydrochloric acid; 1 ml of water, employed to rinse the inside of the crucible cover, was added to the solution, which was then heated for 20 min at 122° in an autoclave, and the cooled mixture was titrated with 0.1 *N* sodium hydroxide solution delivered (directly into the crucible) from a micro-syringe buret. The titration volume was recorded at pH 4.5; the titration was continued after adding 1 ml of a 20% solution of calcium chloride to the mixture, and the titration volume was recorded at pH 8.5. Each titration value was corrected by subtracting from it the corresponding titration value obtained by carrying a 5 ml sample of "blank" solution consisting of water (in place of urine) plus preservative(2) through the same analytical procedure as described for the diluted urine. Urinary phosphorus and ash-TA were calculated from the corrected titration values on the basis indicated in the preceding paragraph.

Procedure for phosphorus and ash-TA in feces and diet. The sample preparations have been described(2). Approximately 300 mg (accurately weighed) of pulverized product was covered with 5.00 ml of 0.2 *N* sodium carbonate solution in a 20 ml platinum crucible (with cover), dried at 80°, and ignited for 4 hours at 480°. The cooled ash was dissolved in 5.00 ml of 1.2 *N* nitric acid with gentle warming, and the solution was transferred quantitatively to a 100 ml volumetric flask and diluted with water to volume. A 5.00 ml sample of the ash solution was placed in a 20 ml beaker and heated for 20 minutes at 122° in an autoclave. This solution, after cooling, was titrated as described for the urine-ash solutions. Each titration value was corrected by subtracting from it the corresponding titration value obtained by carrying a "blank" through the same analytical procedure as described for the sample. Phosphorus and ash-TA values for feces and diet were calculated from these titration values on the same basis as for urine.

The possibility of losses of mineral elements by volatilization during the ashing procedure was checked with purified salts of known composition. The additional possibility of sulfur losses in the form of organic sulfur was checked by assaying the samples for total sulfur(2) both before and after subjecting them to the ashing procedure. A further check on the possibility of chloride losses was made by assaying the samples for total chlorine both before and after subjecting them to the ashing procedure. Total chlorine was measured as chloride in samples mineralized by combustion in closed Schöniger flasks. Losses of chlorine and sulfur are equivalent to losses of ash-TA value. Losses which could be shown to be significant were therefore accounted for in correction values added to the ash-TA titration values.

Results. Losses of sodium, potassium, calcium, magnesium and phosphorus in consequence of the described ashing procedure could not be detected. Sulfur losses in consequence of ashing the urine samples were generally negligible but were marginally appreciable for urines from rats receiving a daily supplement of 3.00 meq of L-methionine sulfur in the diet. Ashing the latter urines effected a mean (±S.D. of mean for 10 specimens) loss of 0.23 ± 0.02 meq of sulfur per 24-hr sample. Sulfur losses in consequence of ashing the feces samples were nearly negligible (0.15 ± 0.02 meq per 24-hr specimen for 15 representative pooled samples) for feces from animals on a semi-synthetic diet (1) but were considerable (0.41 ± 0.03 meq per 24-hour specimen for 23 individual samples) for feces from animals maintained on Purina Laboratory Chow Meal. Significant chloride losses in consequence of ashing either urine or feces as described could not be detected; however, ashing the Laboratory Chow Meal diet in absence of added sodium carbonate† effected chloride and sulfur losses of (mean ±S.D. of mean for 10 replicate ashings) 0.40 ± 0.01 and 1.29 ± 0.01 meq, respectively, per 8.00 g (24-hr ration per rat) of diet.

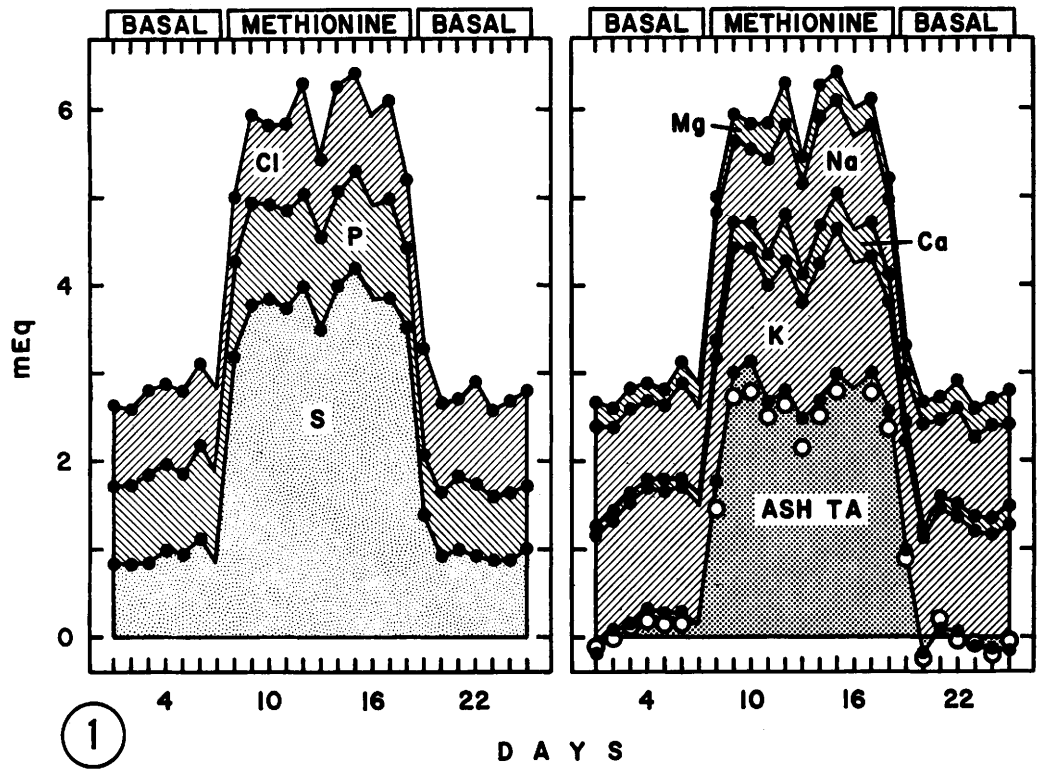
Distribution of anion-forming (Fig. 1, left) and cation-forming (Fig. 1, right) mineral elements in consecutive 24-hr urines of a rat maintained on 8.00 g of Purina Laboratory Chow Meal per day with and without a daily supplement of 1.50 mmol L-methionine are shown in Fig. 1. Values for the anion-forming elements (Fig. 1, left) are plotted consecutively upward, so that the top curve indicates total values. The latter (top) curve is reproduced in Fig. 1, right, and consecutive values for cation-forming elements are plotted downward from it, so that the lowermost curve (Fig. 1, right) indicates total values for anion-forming elements minus total values for cation-forming elements. This (the lowermost) curve therefore indicates ash-TA values calculated from separately determined mineral values. Titrimetrically determined ash-TA values are shown (larger open circles) on the same curve (Fig. 1, right) for comparison. The mean (±S.D.) discrepancy between the urinary ash-TA values measured titrimetrically and those calculated from separately determined mineral values was 0.09 ± 0.13 meq/24-hr specimen.

Data analogous to those in Fig. 1 but derived from consecutive 24-hr feces samples are shown in Fig. 2. The fecal cation-forming mineral elements are shown in Fig. 2, left, and the anion-forming mineral element values (Fig. 2, right) are plotted consecutively downward from the total cation-forming mineral element values, so that the lowermost curve in Fig. 2, right, corresponds to negative ash-TA values calculated from separately determined mineral values. The mean (± S.D.) discrepancy between the fecal ash-TA values measured titrimetrically (larger open circles, Fig. 2, right—the values shown have been corrected for losses of sulfur on ashing) and those calculated from separately determined mineral values was 0.14 ± 0.12 meq/24-hr specimen.

The mean (±S.D. for 5 representative samples) discrepancy between Purina Laboratory Chow Meal ash-TA values measured titrimetrically (corrected for chloride and sulfur losses during ashing) and those calculated from separately determined mineral values was 0.20 ± 0.04 meq/8.00 g (24-hr ration) of diet sample.

Discussion. The generally satisfactory agreement between ash-TA values determined titrimetrically and those calculated from separately determined mineral values indicated that the described procedure would be adequate for establishing the relationships between ash-TA values and renal "net acid" values which have been examined and described elsewhere(1). It is of interest that a procedure analogous to that described in this report for titrimetric determinations of ash-TA was employed by Davidson and Leclerc(5) for measuring "acid-base balance" in agricultural products. However, the Davidson and Leclerc procedure titrated phosphate ion in the ash solutions to widely differing extents according to amounts of calcium and

† Omitted, in this instance, to permit accurate determination of sodium (together with other elements) in the same ashes as those employed for the ash-TA titrations.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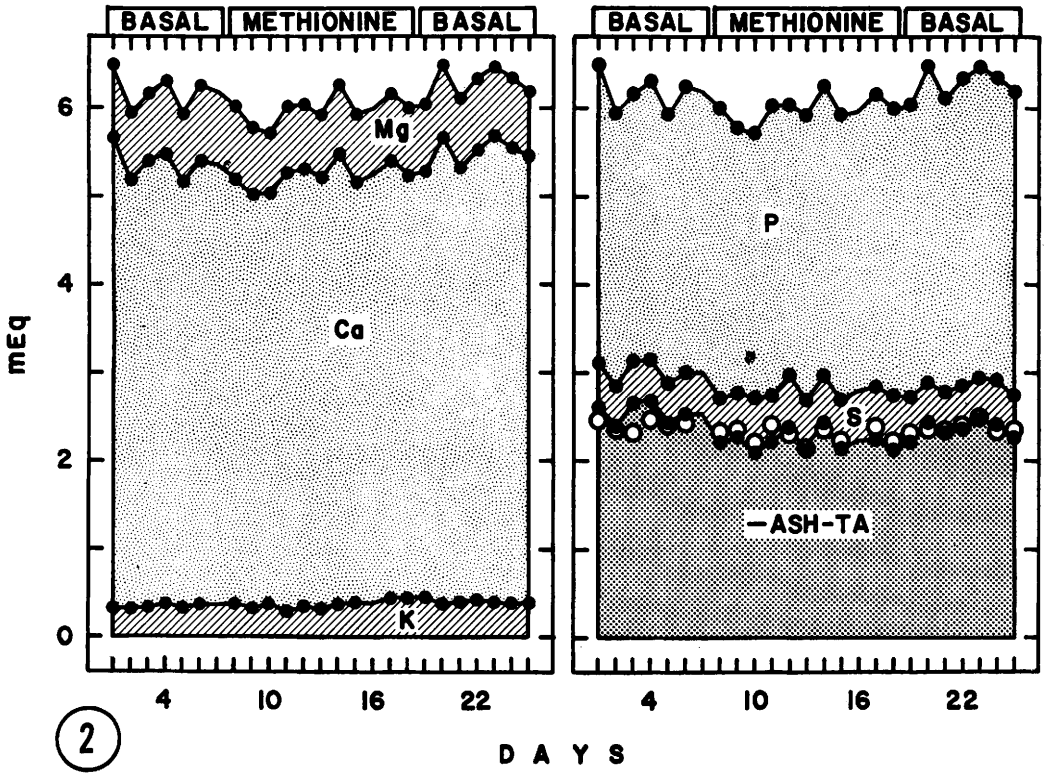
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FIG. 1. Distributions of anion-forming (left) and cation-forming (right) mineral elements in consecutive 24 hr samples of rat urine as described in text. Titrimetrically evaluated ash-TA values are shown as open circles (right).

FIG. 2. Minerals in feces samples corresponding to the urine samples of Fig. 1 (see text). The Na and Cl values (averaging 0.08 and 0.04 meq/24 hr sample, respectively) were too small for individual representation and were therefore included in the representations of the value for Ca and P, respectively. This compromise leaves the latter representations with errors from this source averaging less than 1.5% and retains original accuracy of values shown for total elements and ash-TA.

magnesium present in the samples, so that their titrimetric values could not be reconciled with corresponding values calculated on a uniform basis from mineral composition.

Summary. Titratable ash-acidity (ash-TA) is an acid-base parameter which is defined in terms of mineral composition and may be evaluated as the sum of values for Cl, S and P minus the sum of values for Na, K, Ca and Mg. A relatively simple and essentially direct titrimetric procedure for determining ash-TA in urine, feces and foods is shown to yield values which agree satisfactorily with those calculated from the mineral composition of these materials.

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Sex Related Differences in Isozymes of Serum Lactic Dehydrogenase (LDH).* (32366)

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Electrophoretic studies of the isozymes of serum and tissue LDH have made possible several contributions to the diagnosis of specific organ injury(1-3), and more recently have proved of aid in attempts to clarify the origin of serum LDH(4,5). As will be shown here, they have in addition permitted the discrimination of differences in the distribution of LDH isozymes present in the serum of men and young women. These differences were demonstrable despite the similarity in the mean total serum LDH activity in the two sexes. These new findings indicate that factors related to sex are important physiological determinants of the serum LDH isozyme composition. They also have possible implications, which will be discussed, concerning both the action of sex hormones in controlling the production or assembly of these isozymes and the use of isozymes of LDH in the diagnosis of specific organ disease.

Methods. Venous blood was collected from all subjects in the usual manner, without regard to time of day or meals. Each sample was allowed to clot in a glass tube at room temperature for one-half to one hour before the serum and clot were separated by a 15-minute centrifugation at 2400 rpm. Serum samples were kept at 4°C until used, and were assayed spectrophotometrically and electrophoretically within 24 hours of the blood collection.

The spectrophotometric method for measurement of total serum LDH activity has been described(2,3).

The electrophoretic separation, staining technique, and methods for quantitating the

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