The Mechanism of the Natriuresis of Fasting

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ABSTRACT This study tests the hypothesis that obligatory cation coverage of metabolically generated anions is the mechanism for the sodium diuresis of fasting. Nine obese female subjects were equilibrated on a constant sodium and caloric intake and then fasted while sodium intake was maintained. Particular attention was paid to maintaining the same upright activity schedule during fasting as during control. Consecutive 3-h increases in urinary sodium, ammonium, and potassium excretion during fasting were matched against simultaneously determined increases in organic acid anions (OAS) and $H_2PO_4^-$, which would exist in combination with the cations. The changes were significantly correlated ($r = 0.891$, $P < 0.001$) in the relationship $y = 0.73x + 19$ where y equals increases in organic acid salts + $H_2PO_4^-$ and x equals increases in cations. As ammonium excretion rose, sodium conservation occurred with ammonium replacing sodium at the major urinary cation. Corollaries to the hypothesis were also found to be true. They were: (*a*) Increases in ammonium excretion lagged considerably behind increases in OAS + $H_2PO_4^-$ during the diuretic phase making sodium coverage necessary. (*b*) Sodium loss was much greater than chloride although chloride balance was minimally negative. (*c*) After refeeding with glucose, sodium excretion promptly decreased and appeared best correlated with simultaneous decreases in OAS. Ammonium excretion also fell but much less than sodium. The data support the hypothesis that obligatory cation coverage of metabolically generated anions is a major mechanism responsible for the sodium diuresis of fasting.

INTRODUCTION

Large quantities of electrolyte and water are lost in the urine during the 1st wk of fasting. The principal electrolyte excreted is sodium and the phenomenon has been termed the "natriuresis of fasting." Cumulative negative sodium balance markedly exceeds that which would occur with sodium deprivation alone (1). Also, negative sodium balance occurs even when prefast sodium intake is maintained during fasting (2, 3). Although numerous explanations of this diuresis have been proposed, the mechanism has not been established. Gamble, Ross, and Tisdale in 1923 (4) suggested the role of ketoacidosis, but Gamble later ruled this out in his Harvey Lecture (5). More recently, Bloom and Mitchell (1) have stated that "the ketonuria of fasting is not an adequate explanation for the observed sodium diuresis." Changes in aldosterone and the effects of acidosis either cannot account for the sodium diuresis or have been inconclusive (6, 9). A role of glucagon as a "natriuretic hormone" of fasting has been postulated (3, 10).

This study re-examines the mechanism of obligatory sodium coverage of metabolically generated anions, primarily ketoacids, as a major factor in the sodium diuresis of fasting.

METHODS

Studies were performed on nine obese but otherwise normal, young female subjects in an air-conditioned metabolic research ward. The average weight was 124 kg (range 71.4–159 kg). Each subject was fully informed of the purpose and risks involved in fasting and bladder catheterization, and the study was approved by the Lankenau Hospital Research Review Committee. All subjects had normal blood cell counts, blood sugars, serum electrolytes, urinalyses, blood urea nitrogen, creatinine, electrocardiograms, thyroid function tests, i.v. pyelograms, and negative urine cultures.

Stabilization period. After admission to the metabolic unit, subjects were stabilized for 7–14 days on a regular food (four subjects) or on an electrolyte-free formula diet (five subjects) calculated to keep weight constant. Formula diets consisted of protein (as casein), fat (as corn oil and salt-free butter or lard), and carbohydrate (as glucose) in caloric proportions similar to that previously consumed, as determined from a careful diet history. Sodium was given

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TABLE I

Average Daily Activity Schedule Compiled from a Weeks Observation during Stabilization (Subject P. F.)

	Cumulative pedometer mileage	Walking	Standing	Sitting in chair	Sitting in bed	Lying in bed	Sleeping
8–10 a.m.	¼ mile	1¼ h	15 min	30 min			
10–12 noon	½ mile	30 min		30 min	1 h		
12 noon–2 p.m.	1¼ mile	1¾ h		15 min			
2–4 p.m.	1½ mile	15 min		1½ h		15 min	
4–6 p.m.	1¾ mile	5 min		1½ h	10 min	15 min	
6–8 p.m.	1⅞ mile	5 min	10 min	¾ h	1 h		
8–10 p.m.	2¼ mile	15 min		1¾ h			
10 p.m.–8 a.m.							10 h

in the form of sodium chloride tablets or capsules at meal time. The sodium chloride content of the tablets and capsules was periodically verified by chemical analysis. Sodium intake for each subjects is indicated in Fig. 2. Subjects on formula diets received 50 meq of potassium a day. When regular diets were fed, a diet history was taken and patients placed on the same daily diet for at least 1 wk before admittance and then for at least 10 days after admittance. All meals for a given study were identical in composition and weight (to the nearest gram) and were prepared simultaneously in advance of the study. 24-h urine specimens for sodium were obtained, and fasting studies were not begun until caloric and sodium balance was stable. Fluid intake was constant for a given patient at 1,500–2,000 cm^3 per day. Formula and regular diets were homogenized, digested, and analyzed frequently to verify electrolyte content.

Activity during the control period was carefully assessed since we have observed that fasting subjects become inactive and recumbent which could lead to a significant sodium diuresis of recumbency and an alteration in the basic 3-h sodium excretory cycle (11, 12). Activity was measured by attaching a pedometer to the subject and recording daily distance walked. In addition, periods of recumbency, periods of sitting, and periods of quiet and standing, were recorded so that an average daily activity-rest schedule could be compiled for each subject. An example of such an average schedule is shown in Table I. This average schedule was rigidly followed during the final days of stabilization and during fasting. On the last day or two of the stabilization period starting at 6 a.m., consecutive 3-h urine collections were started via an indwelling Foley catheter and continued for 7–8 days. Thus each day was divided into eight 3-h collection periods. To avoid breakdown of chemically labile keto acids, other organic acids, and to minimize pH changes, the urine collection bag was emptied hourly. The pH was measured and the specimen was frozen for subsequent analyses. Organic acid titrations were done the day of collection or the following morning. Hourly specimens were combined into 3-h pools. The necessity for frequent urine collections will be discussed subsequently. Throughout all periods of the study air washouts of the urine bladder were used when urine flow was less than 30 cm^3 per hour to insure more complete bladder emptying. The bladder was rinsed every 12 h with dilute neomycin solution and sterile distilled water, and urine cultures were obtained pre- and poststudy. No infections occurred during the study and all subjects were free of bacteriuria when examined 1 mo poststudy.

Fasting period. After 24 or 48 h of 3-h urine collections at the end of the stabilization period, fasting was started and continued for as long as necessary to observe a sodium diuresis. This varied from 5 to 7 days. The prefast sodium intake was continued during the fasting period and was administered in tablets or prepared capsules under direct observation of a nurse, over a 45 min period and at the time meals were previously eaten. Potassium could not be given, except to subject J. N., during the 1st wk because of significant nausea. Continuous 3-h urine collections were continued throughout the fasting period. An attendant assured adherence to the activity-rest schedule. Between 1,500 and 2,000 cm^3 of solute-free water was given each day, the amount being constant in a given patient. Blood pressure, pulse, and temperature were taken four times a day. Subjects were weighed at the same time each morning.

Refeeding period. In six studies varying amounts of glucose were fed and 3-h urine and blood collections were continued after ketosis and sodium diuresis were well established.

Blood specimens were drawn daily throughout the study for sodium, potassium, chloride, bicarbonate, calcium and phosphorus. The cumulative blood loss did not exceed 200 cm^3.

Analyses. Urine pH was determined by a Beckman Zeromatic SS-3 pH meter with temperature correction (Beckman Instruments, Fullerton, Calif.). Organic acids were fractionated into organic acid salts and free organic acids by the titration method of Van Slyke and Palmer (13), as modified by Relman, Lennon, and Lemann (14). In this procedure phosphate is removed and urine acidified to pH 2.7. Back titration of urine from pH 2.7 to the original urine pH with 0.1 N sodium hydroxide measures the amount of organic acid existing as the conjugate salt. This is hereinafter referred to as organic acid salt (OAS),[1] which implies that it is organic acid anion bound to fixed cation or ammonia, as opposed to organic acid anion bound to hydrogen ion, i.e., the free organic acid. Titration from urine pH to pH 7.4 measures the free organic acid moiety. The sum of these equals the total organic acid excretion. This distinction must be made in assessing obligatory sodium coverage of organic acids. Correction for the titration of creatinine in the organic acid titration was not considered necessary since only the increments in organic acid salt excretions between control and fasting are used in this study. Creatinine excretion rate, per se, did not change

[1] *Abbreviation used in this paper:* OAS, organic acid salt.

from control during the first 7–8 days of fasting, a finding evident in the data of others (15).

Urine sodium, potassium, chloride, ammonium, calcium, phosphorus sulphate, and creatinine were measured using standard methods adapted to the Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) (16–22). β-hydroxybutyrate and acetoacetate were determined by the method of Williamson, Mellanby, and Krebs (23). Preliminary experiments indicated that bicarbonate excretion in the urine obtained during fasting was negligible, and thus was not determined. Titratable acid was determined by titration of urine from urine pH to pH 7.4 with 0.1 N sodium hydroxide. All determinations were done in duplicate or until replicates agreed to 3%. Serum sodium, potassium, chloride, calcium, and phosphorus were measured, using the same method as for urines. Blood pH and P_{CO_2} were determined in duplicate on venous blood drawn anaerobically in heparinized glass syringes, using an Instrumentation Laboratory blood gas analyzer (Instrumentation Laboratory, Inc., Lexington, Mass.). Serum bicarbonate was determined on the Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.). 24-h urine values reported during the diuretic phase are obtained by pooling of appropriate aliquots of 3-h specimens.

Calculations, definitions, and assumptions. Consecutive 3-h changes in sodium excretion were calculated by subtracting the control rate (microequivalents per minute) for a given 3-h period during the control period from the fasting rate for the same 3-h period of the day during the sodium diuretic phase. For example, the average 9 a.m. to 12 noon excretion rate on the last 2 days of stabilization is subtracted from the 9 a.m. to 12 noon sodium excretion rate on each of the days of the sodium diuresis. The same kind of calculation was made for each of the seven other 3-h periods in each diuretic day. A "diuretic" day is one in which sodium excretion exceeded intake. These 3-h increments in sodium excretion during the fasting period are termed Δ sodium (microequivalents per minute). The change in excretion of organic acid salts, $H_2PO_4^-$,[2] NH_4^+, and K^+ was determined in the same manner and termed Δ organic acid salts, $\Delta H_2PO_4^-$, ΔNH_4^+, and ΔK^+. To smooth out chance variations in base-line control values (since these are subtracted from all other subsequent values) the 3-h values for the last 2 stabilization days are averaged to obtain the final control value which was subsequently subtracted from the corresponding diuretic values. Since the sodium diuresis can accelerate rather abruptly, urines were collected every 3 h to be certain that increments in sodium and other cation excretions were correlated precisely in time with a particular anion. Theoretically it would be possible for sodium to increase primarily during the daytime hours and organic acid salts or phosphate to increase at night in combination with some other cation (i.e., NH_4^+ or K^+).

Titratable acid. In his original analysis of cation and anion balances during fasting, Gamble and coworkers (4) included the effects of titratable acid. Although changes in titratable acid might be expected to result in changes in fixed cation (and hence sodium) excretion, it is not necessary to include increases in titratable acid in the present calculations. Increases in titratable acid would derive from: (*a*) the titration in urine or organic acid salts to yield increases in free organic acids and (*b*) from increases in phosphate ($HPO_4^=$), which can accept a proton by titration from urine pH to pH 7.4. However, neither changes in free organic acid anions nor changes in the titratable phosphate, $HPO_4^=$, are included in the anion column and therefore there is no need to "balance" them off with increments in titratable acid in the "cation" column. Rather, only those species of anions in the urine which either escaped titration by H^+, i.e. those combined as organic acid salts, or could not be titrated further, i.e. $H_2PO_4^-$, are considered, since only these would have the potential for existing in combination with fixed base (sodium, etc.) in the urine. Furthermore, to include directly determined titratable acid along with other cations would introduce a significant error, since titratable acid is significantly overestimated in the presence of high concentrations of ammonium, as reported by Lemann and Lennon (24).

Balance calculations. In calculating the external balance of sodium and chloride, only urinary excretions were subtracted from intake, since stools were infrequent. Fecal losses of sodium in fasting have been shown to be negligible in subjects given sodium supplements (25).

RESULTS

General characteristics. Fig. 1 and 2. All nine subjects underwent a sodium diuresis with a daily negative sodium balance occurring at some point during the 1st wk of fasting. Fig. 1 shows a typical example of the sodium diuresis in which all major cation and anion constituents in the consecutive 3 h periods are plotted. The relationship of sodium excretion to organic acid salt excretion is evident. The general pattern of sodium excretion and its relation to ammonium excretion is shown in Fig. 2. On day 1 of the fast there is frequently a small decrease in both sodium and ammonium excretion. Since prefast intake of sodium chloride was maintained, this resulted in a positive balance of sodium (and chloride). After approximately 48 h, sodium excretion begins to rise at a variable rate ultimately exceeding intake, giving rise to the sodium diuresis and a negative external balance. Ammonium excretion also begins to rise rapidly from the 2nd day. Ultimately sodium excretion begins to fall (the descending limb of the diuresis) as ammonium excretion continues to increase. In two subjects (P. W., and J. N.) followed for longer periods, a second phase of sodium retention was seen starting on day 7 or 8 with a suggestion of zero balance occurring as a third phase later on. These patterns of sodium excretion conform at least to the diphasic pattern (negative

[2] It is evident that although increments in phosphate excretion are written as $H_2PO_4^-$, a small amount of $HPO_4^=$ is also present and the true base equivalence of phosphate depends on the relative proportions of each. The base equivalence of phosphate was determined from the urine pH and the titration curve for $H_2PO_4^-$, (PK_2 of 6.8). Most of the diuretic urines were quite acid, and the titration curve is such that the base equivalence of phosphate in most of the diuretic urines of pH 5.8 or less is close to 1.00. To simplify calculations we have assumed an average base equivalence of 1.04 based on a urine pH of 5.4. The error resulting from this assumption is insignificant. For urine pH greater than 5.9 we have calculated the base equivalence of phosphate in accordance with the urine pH.

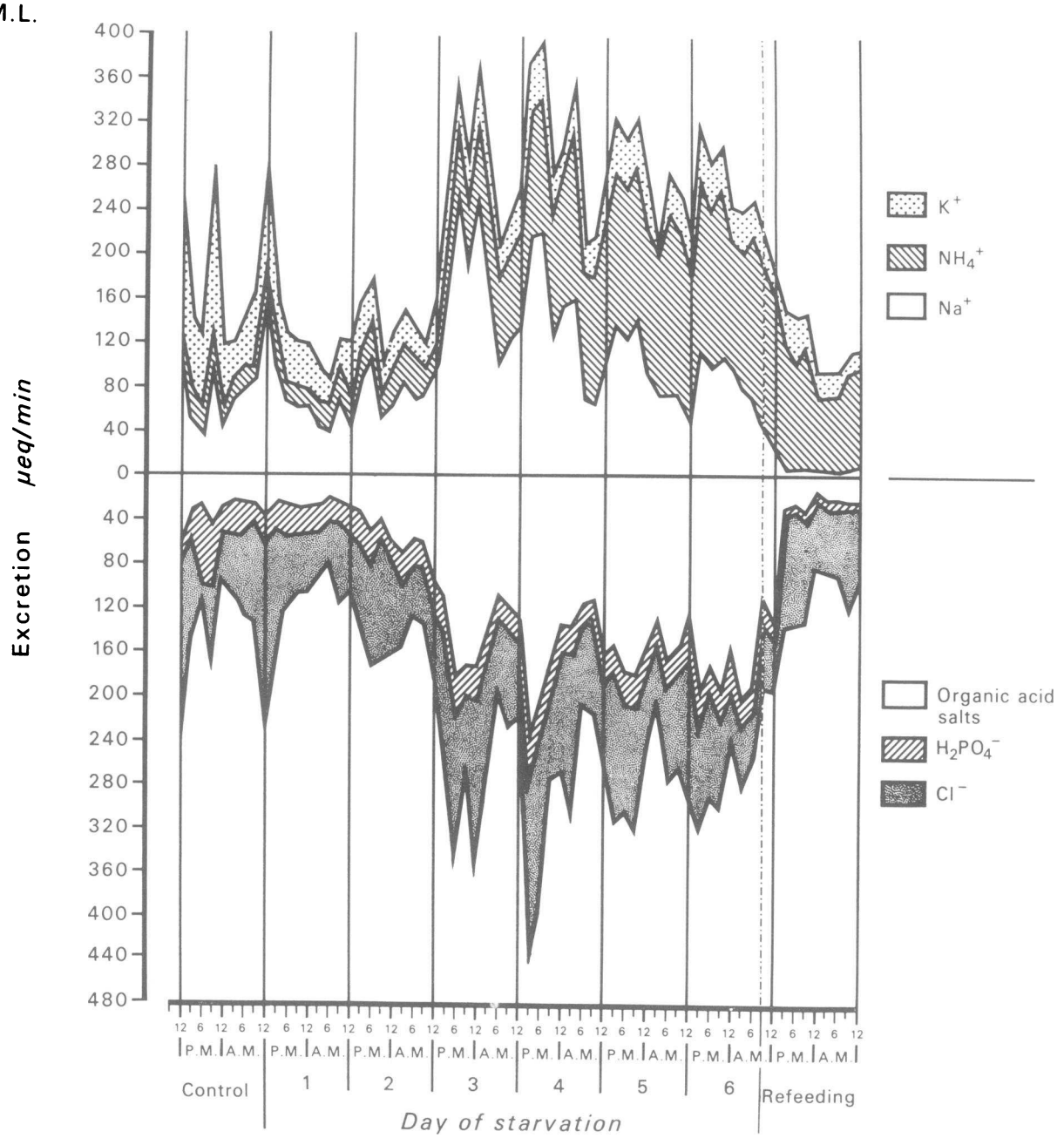

FIGURE 1 Example of the sodium diuresis of fasting. Each point represents the average excretion rate during the previous 3 h, i.e., the point starting at 12 noon represents the 9 a.m. to noon collection. The relationship of the increase in sodium excretion to organic acid salts is evident. Organic acid salts and $H_2PO_4^-$ plateau on day 5 and 6 but sodium falls and is replaced by ammonium. Potassium excretion diminishes slightly.

balance followed by positive balance) observed by Stinebaugh and Schloeder (2) and Boulter, Hoffman, and Arky (3). Most subjects were not fasted long enough to observe the third phase of zero balance. Subjects P. W. and D. C. occasionally vomited a salt tablet. In these instances the sodium content in the vomitus was determined and subtracted from intake as is evident in Fig. 2.

Relationship of increases in cations to anions. (Fig. 3). If obligatory cation coverage of metabolically generated anions is a major factor governing renal cation (and hence sodium) excretion during the diuresis of

fasting, then increments in cations (Δ sodium, Δ ammonia, Δ potassium), as defined above, should be balanced by increments in metabolic anions generated by the fast, i.e., ΔOAS and $\Delta H_2PO_4^-$. These are quantitatively identified by the titration procedure and appropriate phosphate calculations as acid anions that would exist, at a given urine pH, electrochemically attached to base, i.e., the conjugate salts of the acid. When these cationic changes are plotted against the corresponding anionic changes for the same 3-h time period in each diuretic day, a regression line is generated with a slope of 0.73 and a significant linear correlation of 0.891 ($P < 0.001$, $n = 190$). In many instances, except where potassium was fed, (subject J. N.) the value for Δ potassium was negative but the potassium deficit must be included because any decrease in potassium coverage of increasing amounts of $\Delta OAS + \Delta H_2PO_4^-$ would have to be made up by sodium, and this in turn would have the effect of increasing sodium excretion. Although the slope of the regression is close to unity, it

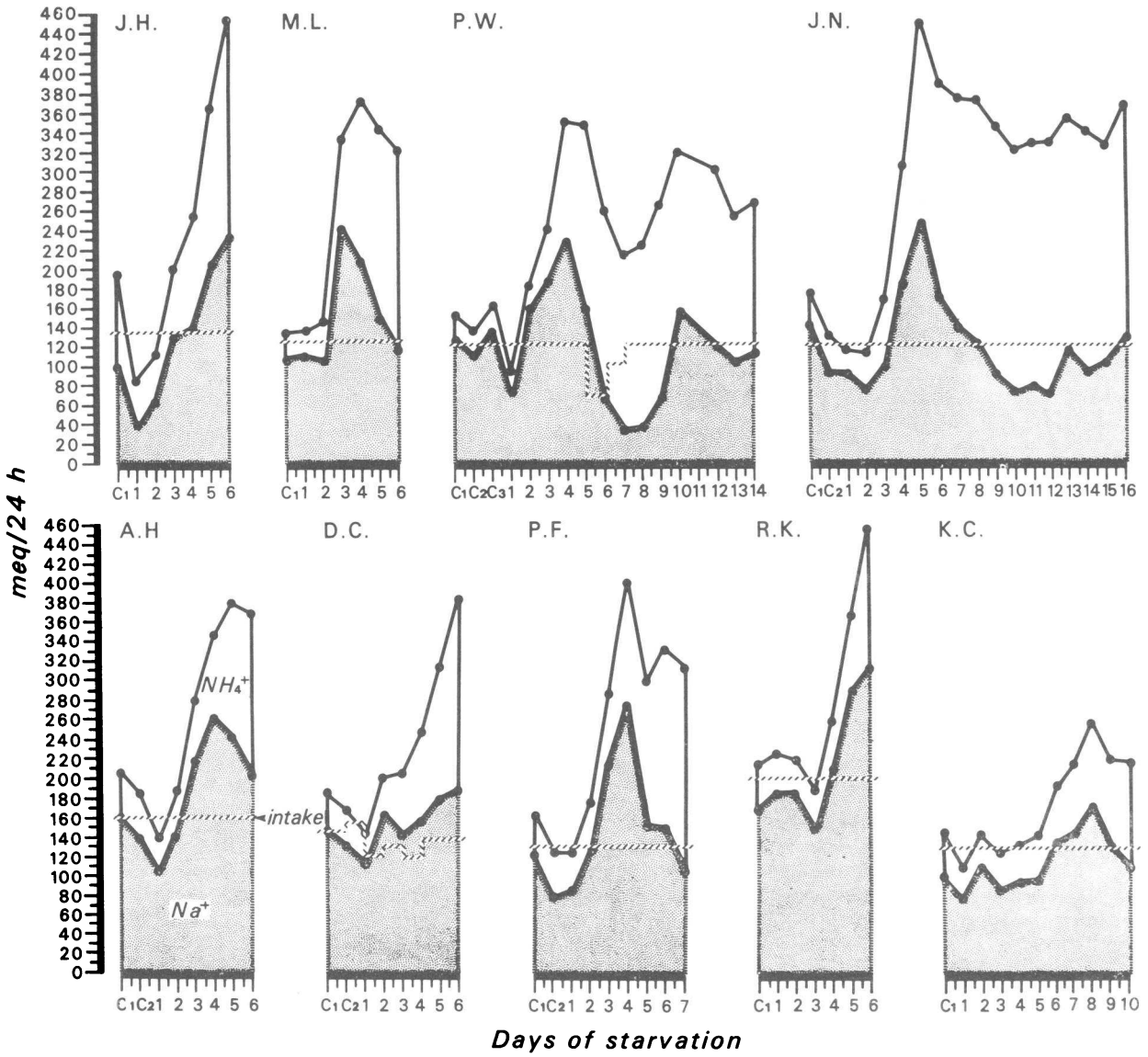

FIGURE 2 Urinary sodium excretion (stippled area) and its relationship to sodium intake and ammonium excretion (open area). The rate of rise of sodium excretion either diminishes or sodium excretion falls as ammonium excretion rapidly rises. Marked increases in ammonium excretion allow sodium conservation to occur. C_1, C_2, and C_3 are terminal days of the stabilization period.

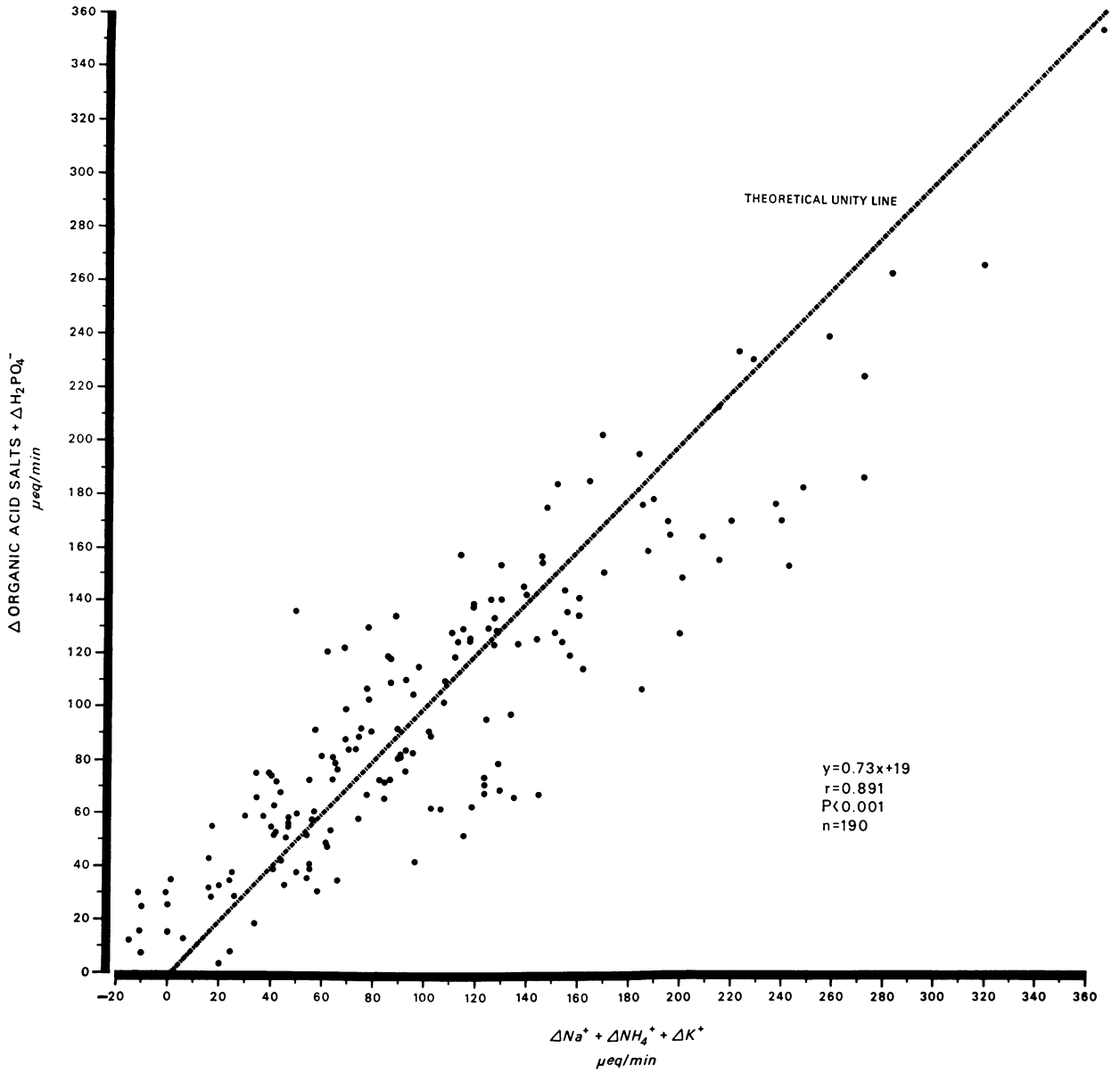

FIGURE 3 Mass plot of data from all subjects relating changes in cation excretion ($\Delta Na^+ + \Delta NH_4^+ + \Delta K^+$) to changes in metabolically produced anions (Δ organic acid salts + $\Delta H_2PO_4^-$). Each point is the calculated change from control of a corresponding 3-h urine collection during diuretic days.

is nevertheless less than unity, which is graphically shown by the fact that some of the points at the upper end fall to the right of the unity line unopposed by similar deviations to the left. This is due to the fact that at very high solute excretion rates increases in sodium and ammonia excretion were also covered in part by small increases in chloride excretion. This may represent a nonspecific osmotic diuretic effect increasing sodium chloride excretion. Changes in calcium and sulphate were determined in several subjects but were of such small magnitude as to be without effect on the curve and are omitted to simplify presentation.

Relationship of increases in ammonium excretion to increases in organic acid salts and $H_2PO_4^-$. Increments in ammonium excretion as well as the simultaneous changes in the sum of the changes in organic acid salts and phosphate excretion during the diuresis are shown in Fig. 4. All points are plotted up from the

zero base line. Increases in ammonium excretion lag considerably behind the corresponding increases in measured anion excretion which would require cation coverage. This difference is depicted graphically by the shaded area and would presumably have to be made up by some other cation, in this case sodium.

Sodium and chloride excretion. Fig. 5 shows the cumulative and daily balances of sodium and chloride during fasting while on the same sodium and chloride intake consumed during the stabilization period. During the first 2–3 days before the onset of organic aciduria the balance of sodium and chloride is positive. Chloride balance tends to become negative during the peak of the natriuresis but very much less than sodium. Excluding subjects J. H. and K. C. whose diureses were delayed until the 5th and 7th days, the mean cumulative negative sodium balance during the 1st wk was 203 meq (range 107–232 meq). The mean cumulative negative chloride balance of 6 meq obscures the fact that on peak diuretic days, daily negative chloride balance was as high as 40 meq/day.

Refeeding studies. Six subjects were fed glucose after the diuresis was established. The time-course of sodium, ammonia, organic acid salts, and chloride excretions after feeding of carbohydrate (glucose) in two representative studies is shown in Fig. 1 and 6. Within a 3–6 h period after refeeding there occurs an abrupt decrease in both sodium and organic acid salt excretion. There is a significant but lesser decrease in ammonium and in chloride excretion during the first 3 h of carbohydrate administration. However, the higher the refeeding dose of glucose, and the longer it was fed, the more pronounced the fall in ammonia excretion. In general, ammonium excretion falls with glucose refeeding but is still well above prefast excretion rates for the first 24 h. This contrasts to sodium excretion which frequently drops well below prefast excretion.

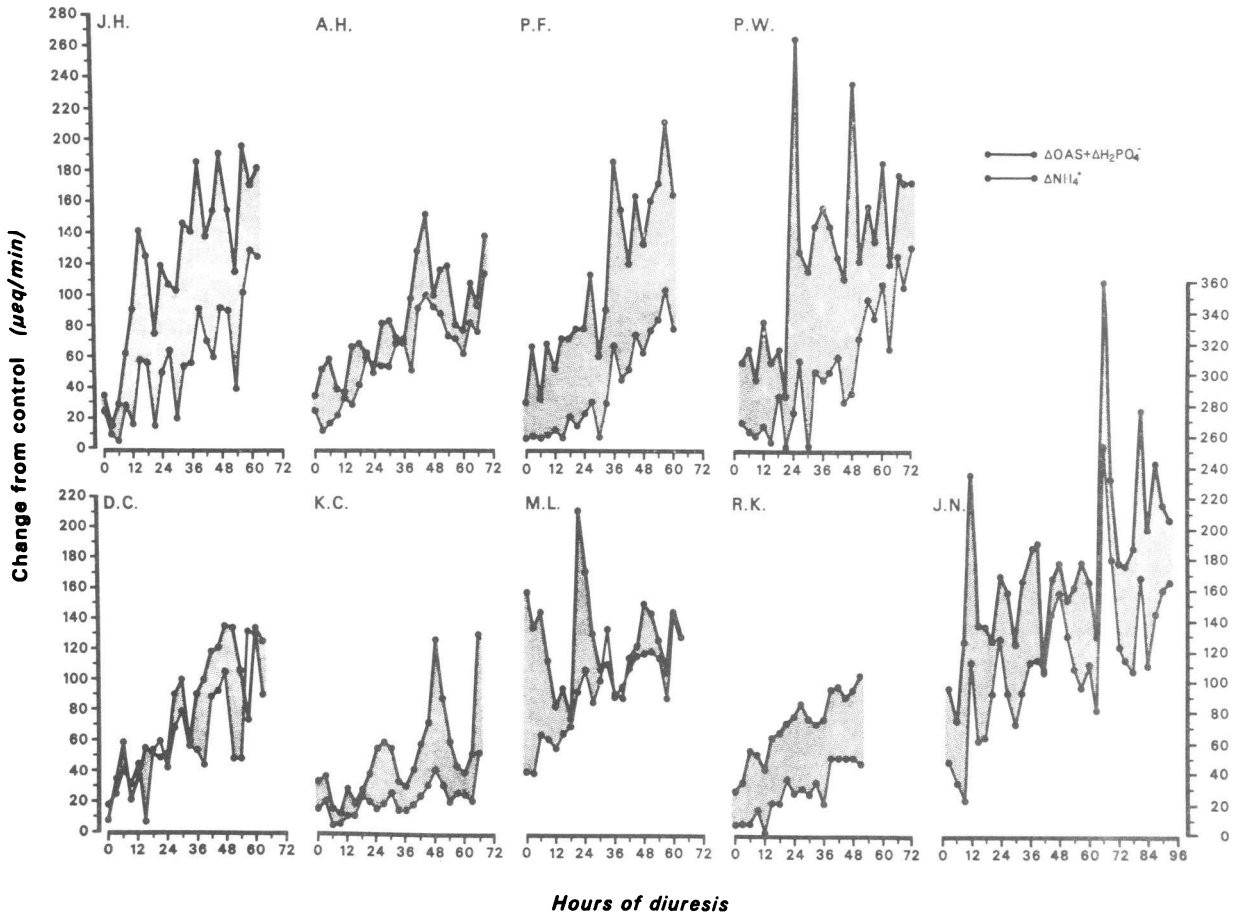

FIGURE 4 3-h increments above control (Δ values) for $OAS + H_2PO_4^-$, compared to ammonium during the diuretic phase. All points are plotted from the vertical zero point. Shaded area is the amount by which increase in ammonium lag behind increases in $OAS + H_2PO_4^-$.

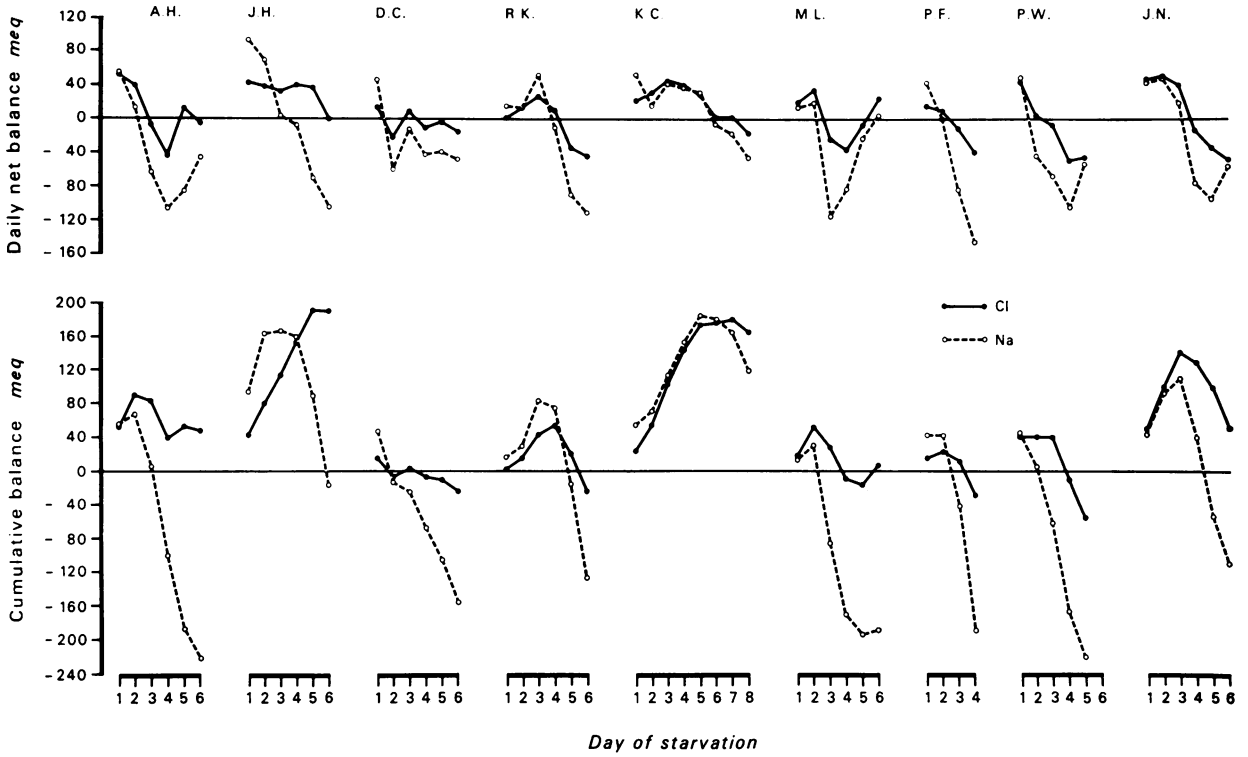

FIGURE 5 Daily and cumulative balances of sodium and chloride calculated by subtracting urine output from maintained prefast intake. The difference between sodium and chloride balance is evident.

DISCUSSION

The results of these experiments describing the pattern of sodium excretion during early fasting are in accord with considerable previous works, (2, 3, 6, 7, 8). However, the present experiments are unique in that they measure changes in sodium and other major cation excretions continuously in 3-h intervals during many days of fasting. At the same time direct measurements are made of simultaneous changes in excretion of weak acid anions which would exist in urine electrochemically associated with these cations, i.e., exist as the conjugate salts of the acid. We are unaware that the quantitation of organic acid salts, as opposed to total organic acids, has been previously reported in this context. This experimental approach affords an opportunity to test the hypothesis of obligatory cation coverage of organic acids. The data thus obtained support the hypothesis that a major factor responsible for this negative sodium balance is obligatory cation coverage (and hence sodium coverage) of metabolically generated organic acid anions and acid phosphate ($H_2PO_4^-$). The role played by $H_2PO_4^-$ is much smaller compared to organic acid salts. Gamble (5) indicated that the increments in total organic acids (presumably keto acids) [3] are exactly balanced by the increases in urinary titratable acids plus ammonium, thus making it unnecessary for sodium to cover the organic acids during starvation. However, in Fig. 4 it is invariably seen that although ammonium excretion rises rapidly above control levels during fasting, the increments in ammonium are initially insufficient to cover the organic acid salts or the acid phosphate moieties, both of which must be covered by some cation. This difference must be made up by sodium since potassium excretion rises vey little, if at all. A similar quantitative lag in the increase of ammonium excretion relative to keto acid (i.e., organic acid) excretion during the first 5–7 days of fasting is evident in the data of Owen and Cahill (27). Conversion of the ammonium excretion data in their Fig. 4 and 5, into meq/24 h and comparing the incremental change in ammonia over control to increases in β-hydroxybutyrate and acetoacetate, show

[3] Gas-liquid chromatographic analysis and chemical quantitation in three subjects confirmed the findings of Sapir et al. (26) that increases in urinary organic acids during fasting are due predominently to increases in ketoacids. Therefore, in this discussion, the terms keto acids and organic acids are used interchangeably.

that milliequivalent increases in ammonium lag well behind β-hydroxybutyrate and acetoacetate as recorded in their Fig. 4 and Fig. 6. Similar findings are evident in the data of Sapir et al. (26). These workers state that the increases in keto acid excretion correlate well and are matched by increases in ammonium excretion. However, during the first 4–7 days of fasting before keto acid and ammonia production have plateaued, (i.e., before a new steady state has been achieved) the rate of increase in keto acid excretion exceeds the rate of increase in ammonium. This is consistent with the biphasic nature of the sodium excretion pattern. Initially, there is sodium loss and then relative sodium gain after ammonium matches keto acid excretion and replaces sodium as the covering anion. The excretion pattern of sodium and its relationship to ammonium excretion (Fig. 2) is very similar to that seen in the sodium diuresis with ammonium chloride-induced acidosis in which obligatory cation coverage of an excreted acid anion (chloride) is a major factor in the sodium loss (28).

The significant linear regression line which is generated by plotting the sum of the 3-h increments in sodium, potassium, and ammonium excretion against the sum of the same 3-h increments of organic acid salts and $H_2PO_4^-$ during the diuretic phase, further supports the obligatory cation coverage hypothesis. The slope is quite close to 1.0 as predicted (0.73) and the correlation ($r = 0.89$) is significant, $P < 0.001$. In order for the points to fall exactly on the theoretical unity line, it must be assumed that the circadian rhythm of excretion present during stabilization for all ions would have remained constant in amplitude and periodicity for the succeeding 7 days had fasting not occurred. This is implicit since these control rates are subtracted from the corresponding diuretic values to obtain the Δ values. Such absolute constancy of underlying circadian rhythm may not always occur and deviations from the ideal curve could in large part be attributable to such shifts. Also, unavoidable and variable chemical decomposition of acetoacetate would account for some deviations.

If, in fact, obligatory cation coverage is a major determinant controlling sodium excretion during the 1st

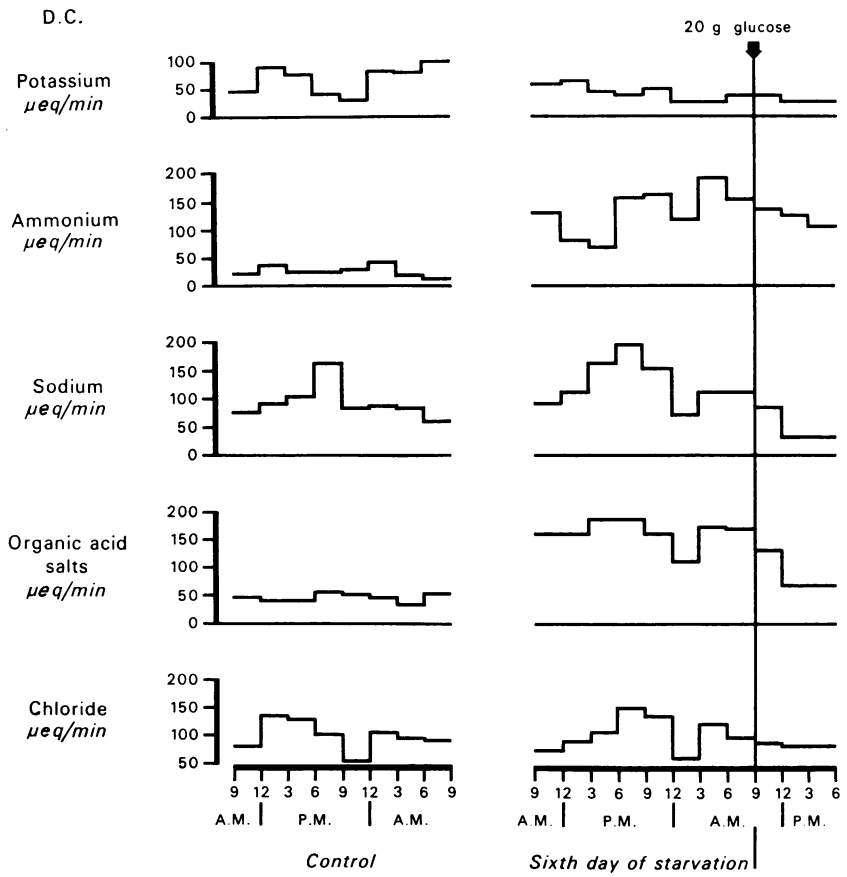

FIGURE 6 Electrolyte excretion after glucose refeeding after fasting. Note the abrupt fall in organic acid salt excretion coincident with fall in sodium excretion.

wk of fasting, then certain characteristics of the diuresis should be evident. Thus sodium and chloride excretion should become dissociated. Fig. 5 shows that cumulative and daily sodium balance clearly diverges from chloride. The daily balances do show however that chloride balance is frequently negative. Schloeder and Stinebaugh in Fig. 1 of their paper (29) shows a relatively minimal increase in chloride excretion during the 1st wk of fasting at a time when sodium excretion is rapidly increasing. In a more recent paper these workers suggest that the pattern of chloride loss follows that of sodium loss (2). However, it is evident in their data that although the pattern or directional change is the same, quantitatively, sodium loss far exceeds chloride loss by at least twofold when comparing their Fig. 5 (sodium loss) with their Fig. 6 (chloride loss).

The fact that cumulative or daily chloride balance is in fact negative at all is of interest and deserves comment. Despite every attempt to maintain the same activity-rest schedule during fasting as during control, fatigue caused some subjects to occasionally lag behind in their prescribed upright activities. Therefore, the small increase in chloride excretion may have been due to increased sodium excretion secondary to the diuresis of recumbency (11). In addition, metabolic acidosis, per se, as it occurs in fasting may have an effect on sodium chloride excretion by inhibiting sodium chloride reabsorption in the proximal tubule. Recent studies suggest that proximal tubular reabsorption of NaCl is linked to the amount of bicarbonate which is present and reabsorbed from proximal tubular fluid (30). Maude (31) in microperfusion studies of single proximal tubules in rat kidney cortex concluded that active transport of $NaHCO_3$ stimulated NaCl reabsorption. As metabolic acidosis develops during fasting and plasma bicarbonate falls, the filtered load of bicarbonate falls. In absolute terms, less bicarbonate is reabsorbed which in turn could result in less proximal sodium chloride reabsorption as would be predicted from Maude's work (31). In addition, the nonreabsorbable fraction of the partially reabsorbable keto acids, as suggested by Schwab and Lotspeich (32), exert an osmotic diuretic effect as they are excreted which further decreases proximal NaCl reabsorption.[4] During refeeding with large amounts of glucose the reverse situation occurs. There is a sharp reduction in concentration of partially reabsorbable keto acids along with a rapid rise within 24 h of plasma bicarbonate (2), both of which could favor proximal reabsorption of sodium and chloride.

A more complete analysis of the mechanism of sodium retention after refeeding with glucose is difficult with the available data. Once the overriding effect of obligatory cation coverage has been abolished by glucose refeeding, multiple other factors causing sodium conservation become operative. These include the effects of extracellular fluid volume contraction on proximal sodium chloride reabsorption, and the effects of increased aldosterone. However, the fact that the decrease in sodium excretion markedly exceeds the decrease in chloride and more closely parallels decreases in OAS suggests that the abolition of the organic acid salt diuresis plays a central role in the diminished sodium excretion.

While this manuscript was in preparation the work of North, Lascelles, and Coates (33) was reported. Their conclusions concerning the mechanism of the sodium diuresis are in agreement with those reported here. However, they base their conclusions on measurements of total organic acids rather than fractionating them into OAS and free organic acids. This would result in an overestimation of anions requiring cation coverage since at the high excretion rates encountered in fasting, total organic acid excretion is significantly greater than organic acid salt excretion. Conversely, they do not include increments in $H_2PO_4^-$ which would underestimate obligatory cation coverage. These factors would tend to cancel each other out, thus leading to the same conclusion reached in our experiments.

[4] Combined filtered loads of the poorly reabsorbable anions, β-hydroxybutyrate and acetoacetate, measured in this study and calculated from others (26), are in the range of 500 μmol/min which is associated with osmotic diuresis (32).

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