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THE RELATION OF SULFUR METABOLISM TO ACID-BASE  
BALANCE AND ELECTROLYTE EXCRETION: THE  
EFFECTS OF DL-METHIONINE IN  
NORMAL MAN \* †

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It has been known for many years that most of the inorganic sulfate in the urine is derived from the oxidation of sulfur-containing organic compounds. In the classical calculation of the "acid-base balance" of food each Mole of sulfur in the ash is assigned an acid equivalence of two, thus assuming its quantitative conversion to sulfuric acid or to some other strong acid or acids which yield a total of two equivalents of hydrogen ion per Mole of sulfur (1).

Until recently, however, there have been virtually no studies of the relationship of sulfur metabolism to acid-base balance. In 1956 Hunt (2) reported that the feeding of methionine or sulfur-containing foods to man increased the excretion of acid in the urine. He suggested that it is chiefly the oxidation of dietary sulfur that determines the urinary excretion of acid. Interest in the relation of sulfur metabolism to the acidification of the urine has also been stimulated by the observation that administration of methionine is an effective technique for maintaining an acid urine in some patients with chronic urinary tract infection (3). Small reductions in plasma CO<sub>2</sub> content have been observed in subjects given large doses of methionine (4), thus further suggesting a connection between the metabolism of organic sulfur and acid production.

Although on theoretical grounds the release of two hydrogen ions would appear to be an obligatory consequence of the biological oxidation of methionine-sulfur to sulfate in an aqueous medium, there is at present no experimental demonstration of this relationship. No detailed or quantitative

information about the interrelationships of changes in systemic acid-base balance, urinary excretion of acid and electrolytes and the metabolism of organic sulfur is currently available. The present work was therefore designed to clarify this problem by carrying out balance studies of the effects of large loads of DL-methionine in normal men.

#### METHODS AND MATERIALS

Five balance studies were performed on three healthy young adult males who carried on their usual daily activities during the period of observation. Each study was preceded by a two to four day period of adaptation to the standard diet. The balance then began with a control period, followed by a period of DL-methionine loading, and a recovery period, each lasting five to six days.

Liquid formula diets were utilized, which provided 35 to 40 calories per Kg. body weight and 70 to 90 Gm. protein per day. A soybean flour<sup>1</sup> was used as the protein source, and had the following composition:

Protein	50 per cent
Fat	1 to 2 per cent
Carbohydrate	30 to 35 per cent
Calcium	0.2 to 0.4 per cent
Phosphorus	0.5 to 0.7 per cent
Potassium	57 mEq. per 100 Gm.
Sodium	< 1 mEq. per 100 Gm.
Chloride	< 1 mEq. per 100 Gm.

Corn oil and glucose served as sources of fat and carbohydrate, respectively. In three balance studies 200 mEq. sodium chloride was provided daily in the diet, while in two studies no sodium chloride was added. The subjects drank a constant amount of tap water and took one multiple vitamin capsule each day. Some subjects took small amounts of instant coffee either mixed with the diet or in the supplementary water, and in these cases the coffee was included in the diet analyses.

Since the soybean flour contained only 0.7 Gm. methio-

<sup>1</sup> "Toasted Soy Protein" granular flour, kindly made available by Soybean Division, General Mills, Inc., Minneapolis, Minn.

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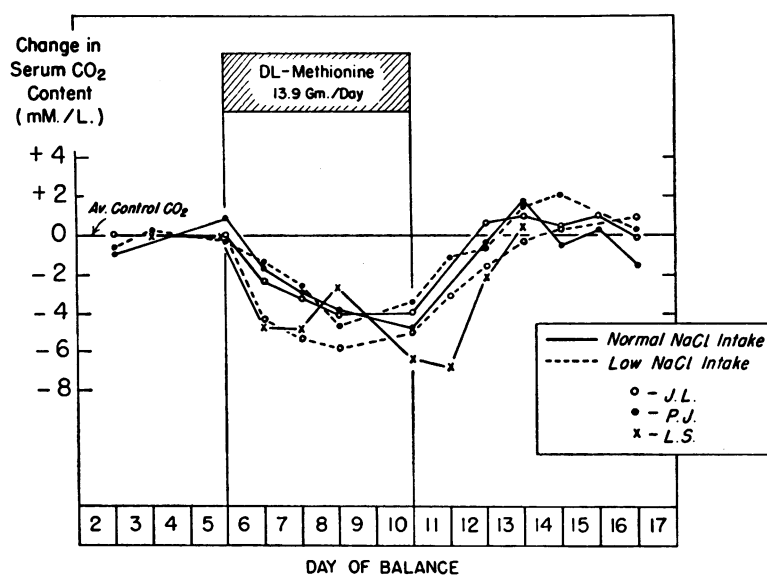


FIG. 1. INFLUENCE OF METHIONINE FEEDING ON SERUM CO<sub>2</sub> CONTENT IN FIVE EXPERIMENTS ON THREE NORMAL SUBJECTS

nine per 100 Gm., a supplement of 1.0 Gm. DL-methionine per day was provided in the basic diet in order to meet minimum daily requirements. An additional 13.9 Gm. DL-methionine per day was added to the basic diet during the period of methionine loading.

Urine was collected under mineral oil using thymol and phenyl mercuric nitrate as preservatives.

Diets were analyzed for total nitrogen, sodium, potassium, chloride and phosphorus. The methods for these determinations, as well as those employed for the analyses of blood, urine and stool have been previously reported (5) with the following exceptions: urinary ammonia was determined by Conway's micro-diffusion method (6), ethereal sulfates in urine were determined gravimetrically as barium sulfate (7) and chloride in serum, urine, stools, and diet was determined by a potentiometric titration (8). Urine pCO<sub>2</sub> and bicarbonate were calculated from the total CO<sub>2</sub> content and pH of the urine. Net urinary excretion of acid (referred to as "net acid") was calculated as the sum of ammonia plus titratable acid minus bicarbonate.

#### RESULTS

Analytical data from a representative balance on one subject (J. L.) are presented in Table I, and some of the pertinent results from the other balances are shown in Figures 1 through 4.

##### I. Effects of methionine loading

In each of the balance studies the administration of DL-methionine produced an immediate though modest drop in serum CO<sub>2</sub> content (Figure 1),

but venous pH did not change. There were no detectable changes in the serum concentrations of sodium or chloride. Potassium concentration decreased by 0.3 and 0.7 mEq. per L. in the two balances without added salt.

Excretion of inorganic sulfate increased rapidly from control levels of 33 to 49 mEq. per day up to 150 to 179 mEq. per day by the second or third day of methionine administration. The maximum daily increments above the average control rates ranged from 110 to 130 mEq. per day. Excretion of ethereal sulfate was measured in two balances and did not change (Table I).

Associated with the sulfate diuresis was a prompt fall in the pH of the 24 hour urine collections to the range of 4.7 to 5.2 (starting from control values of 5.8 to 7.0). The pH of individual freshly voided specimens varied from 4.5 to 5.5 throughout the day. There was also an immediate and progressive rise in ammonium excretion starting from control rates of 19 to 37 mEq. per day and rising gradually to levels of 92 to 115 mEq. per day by the fourth or fifth day of loading. Titratable acid increased by an average of 15 to 22 mEq. per day and remained fairly constant throughout the period of loading. The excretion of "net acid" rose gradually and then leveled off after two or three days. As shown in Figures 2 through 4, the increments in "net

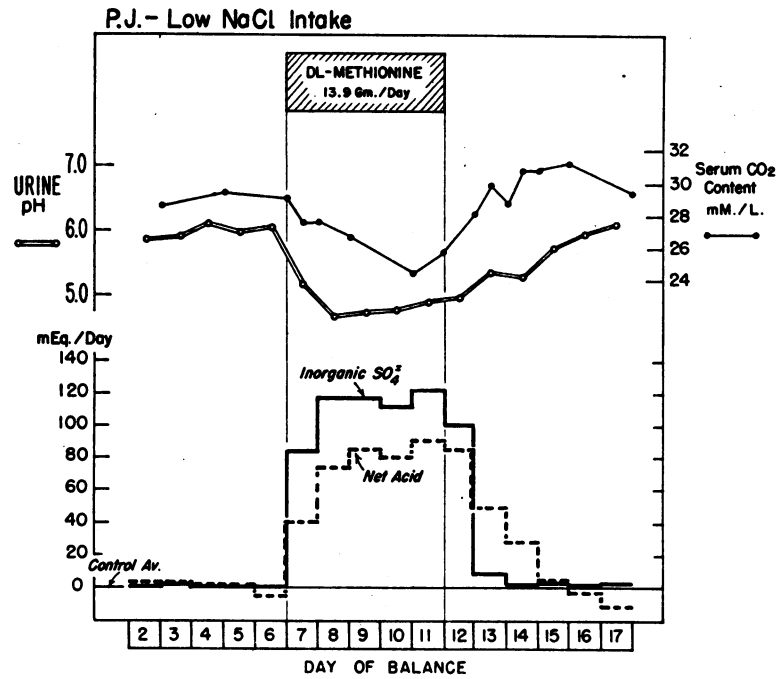


FIG. 2. SUBJECT P.J. LOW SODIUM INTAKE

Effect of methionine feeding on urine pH, serum CO<sub>2</sub> content and on the renal excretion of inorganic sulfate and "net acid."

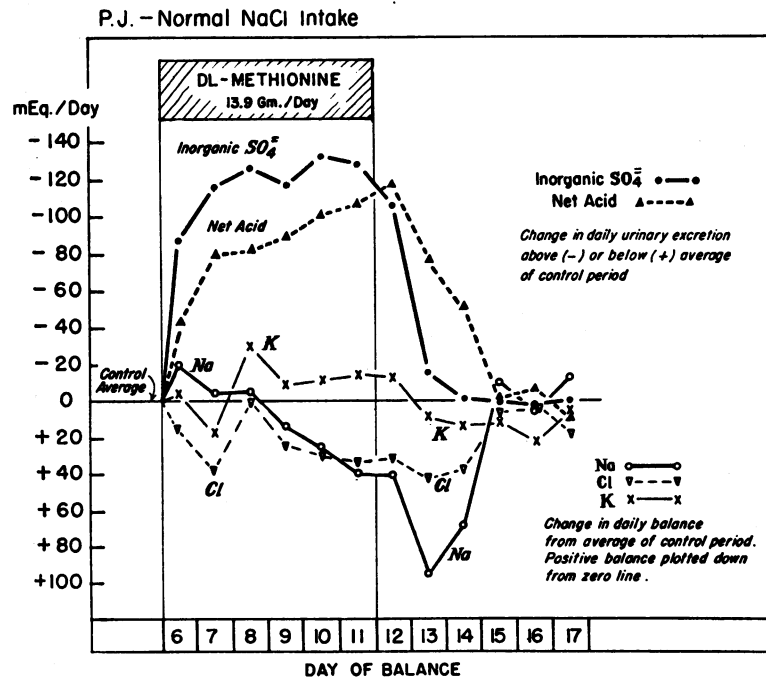


FIG. 3. SUBJECT P.J. NORMAL SODIUM INTAKE

Effect of methionine feeding on the daily balances of sodium, potassium and chloride and on the renal excretion of inorganic sulfate and "net acid."

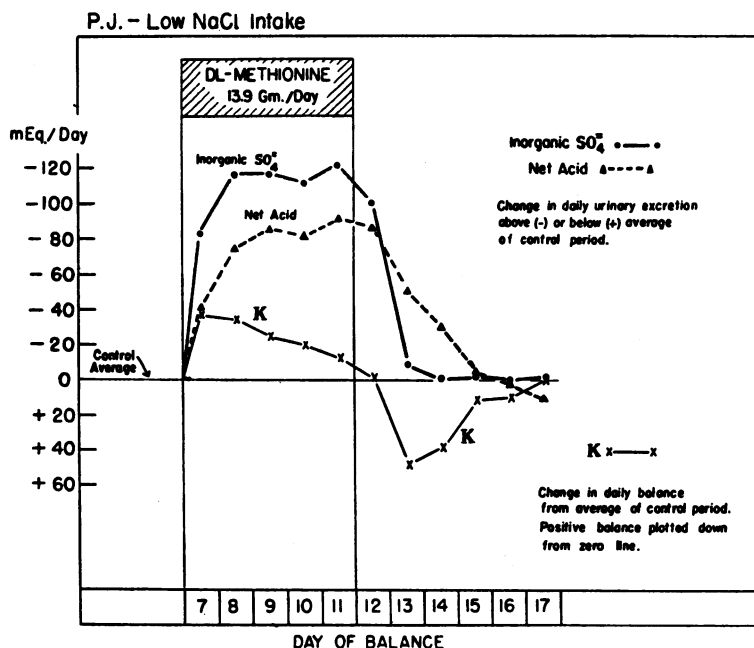


FIG. 4. SUBJECT P.J. LOW SODIUM INTAKE

Effect of methionine feeding on daily balances of potassium and on renal excretion of inorganic sulfate and "net acid." Sodium and chloride balances are omitted because renal excretion was always negligible.

acid" tended to lag behind the increase in inorganic sulfate.

There was no significant or consistent increase in excretion of sodium. In the two low-sodium balances urine sodium and chloride were negligibly small throughout the study. Of the other three balances with added salt, one (Table I) showed no significant change in sodium excretion, another a very small increase in sodium on the first day followed by subsequent retention (Figure 3), and the third a slight initial retention of sodium followed by apparently random fluctuations in balance. Cumulative sodium balances during the loading period, corrected for the control period, were +67 mEq., +50 mEq. and -26 mEq., with corresponding chloride balances of +84 mEq., +136 mEq. and +22 mEq.

Potassium excretion increased slightly in the balances with added salt, usually toward the end of the methionine period (Table I, Figure 3). Cumulative potassium balances, corrected for the control period, were -55 mEq., -49 mEq. and -84 mEq. In the two studies on a salt-poor diet the potassium diuresis was prompt and of greater magnitude (Figure 4). Cumulative bal-

ances for the period, corrected for control, were -111 mEq. and -131 mEq.

Nitrogen excretion in the urine increased in each subject during the loading period by an amount greater than the increment in nitrogen intake represented by the methionine load. Thus, in every instance nitrogen balance became slightly negative during this period (-8 to -15 Gm., total).

Urinary calcium excretion was measured on several days in each metabolic period during three of the studies. It increased from control values of 1 to 3 mEq. per day to levels of 4 to 7 mEq. per day during the period of methionine loading (Table I). There were no marked or consistent changes in excretion of phosphorus.

Excretion of total organic acids showed a small but consistent increase in every study. The average increment above control ranged from 2 to 9 mEq. per day.

## II. Recovery period

Immediately upon stopping methionine, the excretion of inorganic sulfate dropped sharply and

in every instance was virtually back to control levels by the second day of the recovery period. By contrast, however, the excretion of ammonium and titratable acid and the calculated "net acid" excretion remained significantly elevated for two or three days, usually returning to control by the fourth day of recovery. Therefore, as shown in Table I and in Figures 2 through 4, the increment above control in the excretion of "net acid" always exceeded the increment in the excretion of inorganic sulfate on the second and third days of recovery. During this time urine pH remained low, returning to control levels imultaneously with "net acid." As shown in Figure 1, serum CO<sub>2</sub> content was still slightly below control on the morning of the second day of the recovery period but was restored to normal one day later.

In the three studies with added sodium chloride there was a striking retention of sodium immediately after methionine administration was stopped, during the phase when the increment in the excretion of "net acid" exceeded that of inorganic sulfate (Table I, Figure 3). In each instance this positive balance of sodium was accompanied by a reduction in urine volume and a gain in weight of from 0.2 to 0.9 Kg. The cumulative sodium balances for the recovery period, corrected in each instance for the average daily balance during the control period, were: J.L., + 266 mEq.; P.J., + 180 mEq.; and L.S., + 210 mEq. At the end of the recovery period the final cumulative sodium balances for the entire study, including the control periods, were: J.L., + 102 mEq.; P.J., + 272 mEq.; and L.S., + 62 mEq. Chloride balances during recovery were also positive, but significantly less so than those of sodium. The corrected cumulative balances of chloride for the recovery period were: J.L., + 143 mEq.; P.J., + 130 mEq.; and L.S., + 115 mEq.

Potassium excretion dropped below control levels in every case within two or three days after stopping methionine, the retention being greatest in the two subjects on salt-free diets who had lost the most potassium during the loading period. Except for one subject (J.L., Table I) who tended to lose moderate amounts of potassium in his stools throughout the balance, potassium balances were essentially restored to normal by the end of each study.

The excretion of calcium, nitrogen and total

organic acids, which had been slightly increased during the loading period, promptly dropped back to control levels.

## DISCUSSION

### *I. Acid-base balance and electrolyte excretion*

These data clearly demonstrate that large loads of DL-methionine produce a slight reduction in serum CO<sub>2</sub> content and a simultaneous acidification of the urine. Urine pH appeared to be closely related to changes in systemic acid-base balance and to be relatively independent of the quantity of sulfate excreted. Large loads of neutral sodium sulfate are known to produce intense acidification of the urine in salt-depleted subjects (9), probably by accelerating sodium-hydrogen exchange in the renal tubule. This mechanism was apparently not of importance in the present experiments, most likely because the sulfate load was relatively small.

Accompanying the acidification of the urine there was a slow progressive rise in the excretion of "net acid," chiefly in the form of ammonium. Although the increments in "net acid" always lagged behind the rise in sulfate, there was no demonstrable sodium diuresis. There was instead a diuresis of potassium (most marked with low-sodium diets) and calcium, and a slight reduction in chloride excretion relative to sodium. In this respect the effects of methionine would at first appear to be different from those of ammonium chloride, an initial sodium diuresis being a constant feature of the action of the latter compound in normal subjects on normal diets. However, no conclusions can be drawn, for there are no adequate data in the literature concerning the effects of ammonium chloride in doses less than 150 mEq. per day, whereas in the present experiments the maximum increments in sulfate were only 110 to 130 mEq. per day.

While the present experiments were being carried out, an independent study was conducted by Kaitz and Kass (10) for direct comparison of methionine with ammonium chloride. In each of two subjects they compared the effects of 150 mEq. ammonium chloride with those of 12 Gm. DL-methionine (which resulted in an average increment in urinary sulfate of 114 to 119 mEq. per day). There was little or no sodium diuresis with

either agent in those dosages. The initial effects of methionine on acid-base balance and renal acid excretion were, in general, similar to those they observed with ammonium chloride and to the effects of methionine reported here. However, urine pH tended to rise slightly with continued administration of ammonium chloride but not with methionine. They also observed a slight increase in phosphate excretion during methionine loading which was responsible for a relatively greater excretion of titratable acid. Their subjects ingested a considerably greater amount of phosphate than that which was fed in the present experiments, which may, in part, explain the negligible effect of methionine on phosphate excretion in these studies.

### II. Relation between sulfate excretion and endogenous acid production

In the classical calculation of the acid equivalence of the diet ash it is implied that metabolism of dietary sulfur leads to the release of two equivalents of hydrogen ion per Mole of sulfur oxidized (1), and in theory such a result should be expected from the conversion of amino acid-sulfur to sulfate. Although it is well recognized that ingestion of sulfur-containing foods leads to increased acid excretion (2), there has never been any proof of the implied quantitative relationship between sulfur metabolism and the endogenous generation of acid. Since almost all of the oxidized sulfur is excreted as inorganic sulfate in the urine (11), and since all fixed acids are presumably excreted in the urine as "net acid," it should be possible in the present experiments to test the validity of the above concept by comparing the accumulated increments in "net acid" excretion with the increments in sulfate excretion after acid-base balance was restored to normal.

Table II compares these cumulative increments in sulfate and "net acid" in each of the studies. The final cumulative increments in "net acid" ranged from 79 per cent to 105 per cent of the cumulative increments in sulfate, with a mean value of 93 per cent. The close correspondence of these values would therefore suggest that the metabolism of methionine to sulfate does in fact yield two equivalents of hydrogen ion per Mole of sulfur so oxidized.

TABLE II  
*Comparison of cumulative increments in sulfate and "net acid"*

Subject	Diet	Cumulative increment in urinary inorganic sulfate excretion	Cumulative increment in net acid excretion	Cumulative net acid as a percentage of cumulative inorganic sulfate
		<i>mEq.</i>	<i>mEq.</i>	%
J. L.	High NaCl	834	874	105
J. L.	Low NaCl	695	686	99
P. J.	High NaCl	833	747	90
P. J.	Low NaCl	666	524	79
L. S.	High NaCl	644	591	92
			Mean	93

A reasonable description of this quantitative relationship between the endogenous production of sulfate and the generation of acid can be constructed from a consideration of the reactions known to be involved in the metabolism of methionine (11). Figure 5 summarizes what now appears to be the major oxidative pathway for methionine and indicates the probable source of the acid. In the initial step, the details of which are not shown, methionine is demethylated and donates its sulfur to form cysteine. Neutral molecules are involved in this step. The remainder of the methionine molecule is oxidized to CO<sub>2</sub>, urea and water, not shown in the figure. Cysteine is oxidized to cysteine sulfinic acid, (pK = 2.1), thus generating the first equivalent of hydrogen. Following deamination or transamination of cysteine sulfinate to  $\beta$ -sulfinyl pyruvate, a second equivalent of hydrogen is produced by the hydrolysis of the  $\beta$ -sulfinyl pyruvate to acid sulfite (pK<sub>2</sub> = 5.2) and pyruvate. The final oxidation of sulfite to sulfate does not yield additional acid. The potential alkali produced in the form of pyruvate is matched by the potential acid in the form of the amino group removed in the conversion of cysteine sulfinate to  $\beta$ -sulfinyl pyruvate. Thus, the net result of the degradation of 1 Mole of methionine or cysteine sulfur to sulfate is the release of two equivalents of hydrogen ion.

### III. Sulfur balance

Approximately 70 per cent of the sulfur fed as methionine was excreted in the urine as inor-



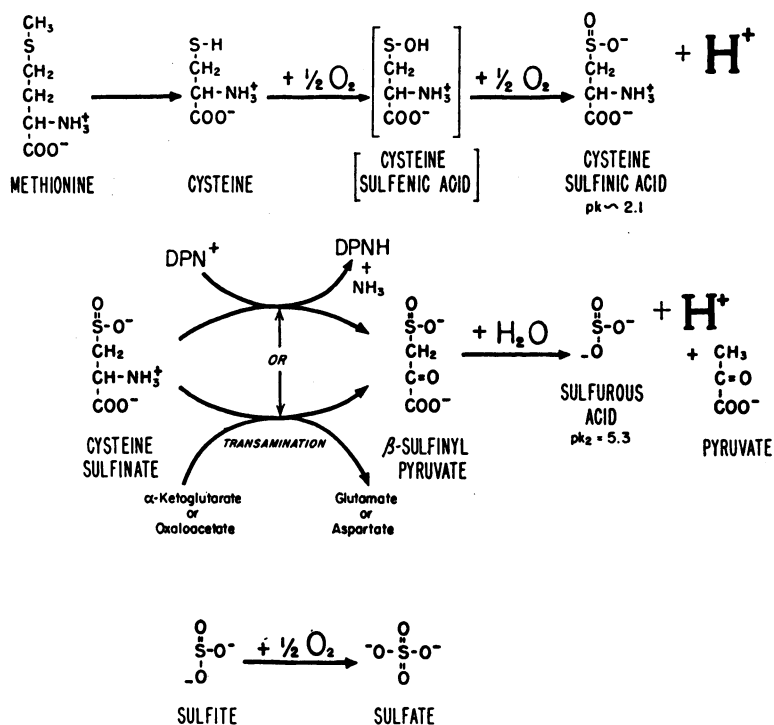


FIG. 5. SUMMARY OF MAJOR OXIDATIVE PATHWAY FOR AMINO ACID-SULFUR  
See text for explanation.

ganic sulfate. There was no increase in urinary ethereal sulfur excretion during methionine loading (Table I) and no increase in the negligible quantities of inorganic sulfate excreted in the feces. Semiquantitative paper chromatographic studies in two balances indicated that an additional 10 to 15 per cent of the sulfur fed was excreted as unmetabolized methionine in the urine. No methionine was detected in chromatograms of the feces.

Thus, roughly 15 to 20 per cent of the administered load of methionine sulfur apparently followed some metabolic pathway other than that shown in Figure 5. Other possible metabolic fates of the sulfur-containing amino acids include: *a*) incorporation into tissue, *b*) deamination to analogous  $\alpha$ -keto acids, *c*) oxidation to taurine and *d*) conversion to volatile compounds such as thiols or hydrogen sulfide. These possibilities are briefly considered below:

*a*) In view of the slightly negative nitrogen balances at the end of each study ( $-1.0$  to  $-17.0$  Gm.) it is unlikely that any significant quantity of sulfur was incorporated into tissue.

*b*) Total urinary  $\alpha$ -keto acids were measured<sup>2</sup> in two balances and were negligibly small, although there was a slight rise during methionine loading. The maximum excretion never exceeded 1 mEq. per day. This suggests that formation of  $\gamma$ -methiol- $\alpha$ -keto butyric acid from methionine was not a major alternate pathway of methionine metabolism.

*c*) Further direct oxidation of cysteine sulfinate yields cysteic acid and finally taurine. No free taurine was found in the chromatograms of the urine in the two balances that were studied. Measurements of fecal taurine and taurocholate excretion were not made. However, in any event, taurine ( $+ \text{H}_3\text{N} - \text{CH}_2 - \text{CH}_2 - \text{SO}_3^-$ ) is an internally neutralized substance and its formation should not affect acid-base balance.

*d*) Volatile sulfur-containing compounds such as methanethiol ( $\text{CH}_3\text{SH}$ ) and methylthiomethane ( $\text{CH}_3\text{SSCH}_3$ ) have been found in the urine of patients with hepatic insufficiency (4, 12) who may have impairment of methionine degradation.

<sup>2</sup> We are indebted to Dr. W. E. Huckabee for these analyses.

Tests for these volatile substances were not made in the present experiments, but several observers noted that the subjects had the characteristic odor of hepatic coma while they were taking the methionine load, thus suggesting that volatile substances probably accounted for at least part of the missing sulfur. However, neither the thiols mentioned above, nor hydrogen sulfide, should have any influence on acid-base balance.

In summary, it would follow from these considerations that : a) most of the administered DL-methionine was metabolized, b) 80 to 85 per cent of the metabolized methionine went to inorganic sulfate, and c) the reactions leading to the formation of inorganic sulfate are the sole source of the endogenous acid derived from sulfur metabolism.

#### SUMMARY

Balance studies were carried out in normal subjects given 13.9 Gm. DL-methionine for periods of five to six days.

Administration of methionine produced small reductions in serum CO<sub>2</sub> content, associated with acidification of the urine and increased excretion of ammonium. There was a prompt sulfate diuresis, the total cumulative increment representing approximately 70 per cent of the administered sulfur, and 80 to 85 per cent of the methionine metabolized. During the loading period potassium excretion increased slightly, but there was no definite change in sodium balance. With recovery, both sodium and potassium were retained.

The changes in acid excretion were related to the observed reductions in serum CO<sub>2</sub> content and to the calculated acid retention. Excretion of acid, on the other hand, was relatively independent of the urine sulfate.

After final restoration of acid-base balance, accumulated increments in sulfate excretion were virtually equalled by the cumulative increments in excretion of "net acid." This strongly suggests the production of two equivalents of hydrogen ion

for each Mole of oxidized sulfur, and confirms earlier views of the important contribution of sulfur metabolism to the endogenous fixed acid load.

Consideration of these data in the light of the known pathways of sulfur metabolism indicates the probable source of the hydrogen ions to be cysteine sulfinic acid and acid sulfite.

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