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Methionine Excretion. Effect of Diet and Methionine Ingestion in Normal Subjects.

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A number of reports[1] in the literature have indicated the outstandingly important role that methionine may play in nitrogen metabolism. Its utilization has been shown to involve not only the intact amino acid but also the sulfur and labile methyl groups in the formation of other essential substances. The varied uses of methionine have been shown by its lipotropic activity and protein sparing action in animals on very low protein diets, and by its role in the detoxification of a variety of organic compounds.[2]

The recently emphasized physiological importance of methionine, along with our earlier studies[3] on animals with fatty livers produced by diets deficient in this amino acid, suggested an investigation of methionine excretion as a new approach to the study of methionine metabolism and as a possible indication of its utilization.[4] Changes in urinary methionine excretion might be caused by increased requirements for this amino acid for essential body proteins. Also increased requirements, which could not be met from available methionine, might be reflected by a decreased excretory response to a test supplement.

Information about the excretion of methionine by normal individuals is scanty. Cleland,[5] in her study of methionine excretion in cystinuria, obtained normal values of the same order as those found by Albanese and coworkers,[4,6] and they both reported an immediate rise in its excretion after ingestion of the free amino acid. If an increase in the protein intake should cause a rise in the excretion of methionine as in the cystinuric,[5] then the effect of fasting and of various dietary regimes, such as low protein, higher protein, and high fat diets, upon its urinary loss should be considered in excretion studies.

Methods. The subjects used in this study were 80 male medical students. The urinary samples for methionine analysis were collected over the 6-hour period, 7 a. m. to 1 p. m., and for the subsequent 18 hours when 24-hour collections were desired. The 6-hour collections were included as a more convenient period of measurement since Albanese[4] has shown a return to the normal level of excretion 3 hours after an intake of 1.5 g of methionine, with equal utilization of the natural and racemic forms. Usually on the following day at 7 a. m., the subjects, fed or fasted, were given 1.0 or 1.5 g of *dl*-methionine orally. The urine collections were started at that time and completed at the end of the same periods used for the controls.

In a second experiment, low protein, higher protein, and high fat diets were followed for 4 days by selecting food from lists of items suitable for such diets. Urine collections were made on the last day of the special diets. Besides methionine, total nitrogen and pH values were determined on these urine samples to indicate how well the diets had been followed. The subjects on high fat diet ingested 20% cream which contains about 2.5 times more ketogenic than antiketogenic substances. This accounts for the fair intake of methionine and only traces of acetone in the urine. They

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1 Lugg, J. W. H., *Ann. Rev. Biochem., Stanford University*, 1945, **14**, 271.

2 Miller, L. L., *J. Biol. Chem.*, 1944, **152**, 603. Also references 1 and 5-7.

3 Treadwell, C. R., Tidwell, H. C., and Gast, J. H., *J. Biol. Chem.*, 1944, **156**, 237.

4 Albanese, A. A., *Bull. Johns Hopkins Hosp.*, 1944, **75**, 175.

5 Cleland, J. B., *Australian J. Exp. Biol. and Med. Science*, 1945, **23**, 273.

6 Albanese, A. A., Frankston, J. E., and Irby, V., *J. Biol. Chem.*, 1944, **156**, 293.

were given a supplement of 1.5 g of methionine and only the 6-hour collections were examined. Separate normal excretion values were obtained on these subjects at this time.

The second experiment was repeated in part on a similar group of students one year later. In addition, 24-hour urine collections, after fasting and a high fat diet, were obtained for examination. Both of these groups developed a marked ketonuria.

In all these studies, total nitrogen excretion values were used to calculate the protein intake of the subjects on the various diets, after allowing 3 days on the diet for stabilizing the plane of nitrogen metabolism. Methionine intakes were estimated by assuming that the protein of the diet contained an average of 3% methionine.[7] The urinary methionine was determined according to the method of Albanese *et al.*[4] Quantitative recovery of methionine added to the urine by us indicates the suitability of the method for the estimation of methionine in urine. However, it must be remembered that the method will respond to any other unknown terminal thio ether or thiocarbonyl group containing substances of the urine. The data were analyzed according to the t method of Fisher[8] and only those results showing a P value of 0.01 or less were considered significant. The standard error of the mean for each value is given with the data.

Results. Our results on the daily urinary excretion of methionine by the adult male compare favorably with those of Albanese *et al.*[4] and Cleland.[5] The former group found an excretion of 247 to 494 mg of methionine per day in a series of 7 observations. Using 80 different subjects, we obtained an average daily excretion of 318 $\pm$ 9 mg (range 199 to 518 mg) or about 4.5 mg per kg body weight.

The methionine excretion during 6- and 24-hour periods and following the ingestion of a 1.0 or 1.5 g supplement is shown in Table I. The latter supplement was approximately one-half of Block's estimate[9] of the average daily intake of methionine. An increased amount of methionine was excreted after the one g test supplement and definitely more after the 1.5 g. However, the percentage of the supplement excreted was the same in both cases, approximately 9% in 6 hours and 15% in 24 hours respectively. An overnight fast caused only a slight change in the methionine excreted, either in the absolute amount of the amino acid, or in the per cent of the test supplement given. Thus, if excretion be taken as a measure, the major part of the free amino acid was utilized (stored or metabolized) even when the intake was markedly increased by the additional methionine ingested.

The remainder of this study was concerned with the effect of fasting and of various diets upon the daily methionine excretion in the normal subject. Three types of diet were ingested—low protein, higher protein, and high fat diets. The total nitrogen of the urines of subjects on these diets served as a check upon the protein intake. Averages of 6.5, 17.7, and 9.3 g of total urinary nitrogen per day, respectively, were obtained in the second experiment. The pH of the urines, the methionine intakes and amounts excreted are included in Table II. Similar data for the third experiment are shown in Table III.

There was no significant difference in the final 24-hour urinary methionine excretions by the subjects on the low and higher protein diets for a period of 4 days. This was true in both experiments on humans (Table II and III), and the observation has been confirmed with rats.[10] These results are supported by the relative constancy of the neutral sulfur excretion.[11] Thus marked variations in the protein content of the diet,† did not affect the daily urinary loss of methionine in these

7 Block, R. J., and Bolling, D., The Amino Acid Composition of Proteins and Natural Foods, C. C. Thomas, Springfield, Ill., 1944.

8 Fisher, R. A., Statistical Methods for Research Workers, 7th ed., Oliver and Boyd, Edinburgh, 1938.

9 Block, R. J., *Yale J. Biol. Med.*, 1943, **15**, 723.

10 Tidwell, H. C., unpublished data.

11 Deuel, H. J., Jr., Sandiford, I., Sandiford, K., and Boothby, W. M., *J. Biol. Chem.*, 1928, **76**, 391.

† Since completion of these studies, a similar observation has been reported by Johnson, R. M., Deuel, H. J., Jr., Morehouse, M. G., and Mehl, J. W., *J. Nutrition*, 1947, **33**, 371.

TABLE I.
Effect of Methionine Ingestion upon Its Excretion.
The fed subjects ingested their normal diets at the usual hours while the fasted subjects had no food for 18 hours including the 6 hours of the urine collection. The normal diets contained approximately 2 g methionine per day.

			Methionine excreted			
			6 hr		24 hr	
No. of subjects	Nutritional state	Methionine supplement, g	total, mg	per kg, mg	total, mg	per kg, mg
11	Fed	0.0	99	1.41	406	5.79
			± 5	±0.07	±13	±0.02
11	”	1.0	185	2.65	550	7.85
			±11	±0.16	±18	±0.28
15	”	0.0	98	1.37	415*	5.73*
			± 5	±0.20	±32	±0.46
15	”	1.5	229	3.16	665*	9.34*
			± 9	±0.15	±41	±0.46
11	Fasted	0.0	83	1.15		
			± 5	±0.07		
11	”	1.5	206	2.85		
			± 7	±0.18		

* Includes 8 subjects.

TABLE II.
Effect of Diet on Urinary Methionine Excretion.
Exp. 2. Subjects ingested the special diets for 4 days. Determinations were made on samples collected during the 6 or 24 hours of the last day. Daily methionine intake was estimated by assuming that the protein of the diet contained 3% methionine.[7]

				Methionine excreted	
No. of subjects	Dietary regime	Urine pH	Calculated methionine intake, g/day	total, mg	per kg, mg
12	Low protein	6.2	1.22	267	3.75
		±0.2	±0.11	±15	±0.19
9	Higher protein	5.6	3.32	252	3.52
		±0.1	±0.06	±19	±0.24
28	Average			257	3.62
				±11	±0.13
9	Normal	6.5	2.10	85†	1.24†
		±0.3		± 5	±0.14
9	” *		2.10	223†	3.11†
			±0.15	±11	±0.15
11	High fat*	5.0	1.74	154†	2.25†
		±0.1	±0.13	± 9	±0.13

* After a 1.5 g test supplement of methionine.
† Determinations made on 6-hr collections.

subjects. The increased methionine excretion after the ingestion of the free amino acid as compared with that after its ingestion in the form of protein might be explained by an increased rate of absorption from the gastrointestinal tract. Fratzer[12] found that the oral administration of hydrolyzed protein produced a greater increase in the blood amino acid level than did the ingested intact protein, and that methionine caused higher levels than did some of the other amino acids. The higher blood levels might account for the increased methionine excretion after the ingestion of the free amino acid.

Although a marked change in the protein intake was without effect on the urinary methionine excretion, a 3-day fast or high fat diet was associated with a significantly decreased excretion as compared with that on

[12] Fratzer, F. H., *J. Biol. Chem.*, 1944, **153**, 237.

TABLE III.
Effect of Diet on Urinary Methionine Excretion.
Exp. 3. Determinations were made on 24-hour samples of the fourth day on these dietary regimes. Daily methionine intake calculated as in Table II.

No. of subjects	Dietary regime	Urine pH	Total N excreted, g/day	Calculated methionine intake, g/day	Methionine excreted total, mg	per kg, mg
13	Low protein	6.6	7.15	1.34	298	4.18
		±0.1	±0.34	±0.07	±14	±0.20
12	Higher protein	6.0	17.74	3.33	290	4.21
		±0.1	±1.29	±0.24	±12	±0.26
12	High fat	5.7	11.88	2.23	252	3.48
		±0.2	±0.97	±0.18	±30	±0.43
9	Fasting	5.1	11.95	0	269	3.42
		±0.0	±0.74		±26	±0.39

the low and higher protein diets used. Also, there was a marked decrease in the urinary methionine after a test supplement was given the subjects on the high fat diet as compared with the normal control values as shown in Table II. Circumstances prevented 6-hour high fat control values on these subjects, but a decreased loss of methionine was found in a 24-hour test in a similar group in the third experiment. Hence a lowered base level of excretion, and a possible slower rate of absorption, might account for a part of the decreased excretion of the supplement in these subjects. However, it is evident that a very high fat intake or fasting, accompanied by a ketonuria, was associated with a decrease in methionine excretion. Increased needs for methionine for lipotropic requirements at times of excessive fat metabolism, owing to a very high fat intake or the excessive use of body fat during fasting, might account for the decreased excretion in these subjects. In this case greater body needs appear to be followed by a decreased excretory response.

The excretion of less methionine by the subjects on the special diets as compared with that of the others in this study was associated with smaller urine volumes during warmer weather. The urines for the dietary studies were collected around May 30 when the average temperature was above 80°F, while the other values were obtained during February and March with temperatures averaging near 60°F. Lack of other known variables than the temperature suggested possible losses of methionine through channels other than the kidneys. Nevertheless, we were unable to correlate the daily urine volumes and the urinary methionine excretion in the various experiments.

Another interesting fact was noted when the per cent retentions of methionine on the different protein intakes in the last two experiments were compared. Values of 78% retention on the low, and 92% on the higher protein diets were almost identical in the last two experiments. This decreased loss in the latter case suggests an increased utilization of methionine with the higher protein intakes.

Summary. Observations on a series of 80 normal male subjects, on a normal control diet, indicated an average daily urinary excretion of approximately 318 ± 9 mg of methionine. The increase in methionine excretion during the 6- and 24-hour periods following the ingestion of a 1.0 or 1.5 g supplement of methionine in addition to that of the usual protein intake only amounted to approximately 9 and 15% respectively of the intake of the free amino acid.

The ingestion for 4 days of diets of varying protein content showed no change in the daily urinary methionine excretion as a result of such variation. A decrease in the methionine normally excreted occurred when the subjects were on a high fat diet or when, on a 6-hour test, they were given a similar test supplement of methionine. Increased lipotropic requirements might account for the decrease in the loss of methionine after a 3-day fast or high fat intake.