Protein Requirements of Man: Variations in Obligatory Urinary and Fecal Nitrogen Losses in Young Men [1,2]

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ABSTRACT For 14 days 83 healthy Caucasian male university students, 18 to 26 years of age and 58 to 104 kg body weight consumed daily either an essentially protein-free diet (44 subjects) or one supplying 0.1 g egg protein per kilogram body weight (39 subjects). Body cell mass (BCM) was calculated from whole body ^{40}K in 37 randomly chosen subjects. Urine was analyzed daily for nitrogen (N) and creatinine; fecal N was measured on pooled samples. Urinary N output reached a steady state between days 3 and 8 for all subjects; the average for days 10 to 14 was taken as the measure of obligatory urinary N loss. Mean urine and fecal N losses were not significantly different for the two diet groups and the data were combined for overall analysis. Obligatory urinary N was normally distributed, averaging 37.2 ± 5.5 mg N/kilogram body weight, 76.8 ± 12.5 mg N/kilogram BCM, 1.8 ± 0.30 mg N/basal kilocalorie. Obligatory fecal N was 9 ± 2 mg N/kg body weight, amounting to 20% of the total obligatory N loss. Although statistically significant correlations were found between obligatory urinary N and body weight, BCM, basal metabolic rate, and creatinine, they accounted for little of the variation in daily loss among individuals. Four subjects were restudied after a 3-year interval; obligatory urinary N loss per kilogram body weight did not differ significantly between the two periods. J. Nutr. *102:* 1595–1604, 1972.

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The minimum nitrogen (N) requirement for an ideal dietary protein in healthy adults has been assumed to be the sum of their urinary and fecal N losses, estimated after adaptation to an essentially N-free diet, plus integumental minimal sweat losses (1). It was recognized that in ordinary life there are many causes of stress sufficient to increase urinary N losses, and an arbitrary 10% allowance was added to take this into account. Similarly, the additional protein N required for growth, pregnancy, and lactation was estimated. The most recent recommendations of the U. S. Food and Nutrition Board also utilize this approach in their assessment of dietary protein allowances (2).

The factorial approach was handicapped by the inadequacy of the data on obligatory N losses in man. The applicable information in arriving at these recommendations (1, 2) was derived from studies by Martin and Robison (two men) (3); Smith (4) and Deuel et al. (5) (one man each); Mueller and Cox (four men) (6); Hawley et al. (six women and seven men) (7); Kofrányi (two men) (8); and Murlin et al. (five men) (9). In addition, obligatory N excretion data were available for a total of 34 young women from studies by Bricker et al. (10), and Bricker and Smith (11). The information published since is

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[2] Contribution no. 1893 from the Department of Nutrition and Food Science, MIT, Cambridge, Massachusetts. A preliminary report of this study was presented at the 52nd Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, April 1968. Federation Proc. 27: 485 (abstr.).

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that of Young and Scrimshaw (12) on 11 young men, Calloway and Margen (13) on 13 young men, and Gopalan and Narasinga Rao (14) on four undernourished but otherwise healthy men in India.

Since the available direct data were inadequate to assess mean values and the extent of individual variation, the 1965 FAO/WHO Committee (1) turned to the review of Smuts (15) which proposed a value of 2 mg N per basal kilocalorie for the relationship between basal metabolic rate (BMR) and obligatory urinary N based on data from five mammalian species. By adopting this value for calculation of human obligatory urinary N loss in the factorial equation, it developed estimates for the protein requirements at all ages and physiological states from available data on BMR. Moreover, the coefficient of variation of 10% accepted for BMR (16) was applied to the presumed variation in obligatory urinary N losses. The fact that the data on human subjects (7, 9, 10) fell below the regression line derived from the other species was merely noted. No quantitative justification was given for further application of the 10% coefficient of variation figure to the sum of the various obligatory N losses.

In view of the importance of accurate knowledge of human protein requirements and the increasing use of the factorial approach to estimate them, there is a need for data on the obligatory N losses in a large enough number of subjects to give an accurate estimate of the mean and standard deviation for various populations and to determine whether these vary in different parts of the world. The present study makes available information for 83 young adult male university students of North American or European origin.

EXPERIMENTAL

Subjects. The subjects were Caucasian male MIT undergraduate and graduate students ranging from 18 to 26 years of age, who were free of any abnormality detectable by careful medical history, physical examination, hematocrit and hemoglobin determinations and measurement of serum glutamic-oxaloacetic acid transaminase as an indicator of liver function. The subjects continued their usual activities but did not participate in competitive sports during the study.

The initial physical and metabolic characteristics of the subjects are given in table 1. The first group entered the study in August 1966 and the last was completed during July 1967.

The subjects were paid and also sincerely interested in the objectives of the study. In prior pilot studies with similar individuals given essentially protein-free diets for up to 21 days, we were unable to detect any measurable changes in CNS function as judged by flicker fusion, reaction time, estimated timed interval and mood scale tests.

Diet. All subjects received a diet which, on the basis of a diet history, provided adequate calories. The diet consumed by the first 39 subjects of the study supplied about 0.1 g of egg protein per kilogram body weight (15.2 mg N per kg per day) while the remaining subjects consumed a diet that was essentially protein-free (6.7 mg N per kg per day). The composition of

TABLE 1

Initial characteristics of 83 Caucasian young men studied for obligatory urinary and fecal N losses

Variable	Mean	Standard deviation	Sample size	Minimum	Maximum
Age, yr	20.9	2.1	83	18	26
Height, cm	178.8	6.1	83	162	195
Weight, kg	73.5	9.3	83	57.9	104.1
Height/weight, cm/kg	2.47	.27	83	1.63	3.05
BMR,[1] kcal/day	1535.6	247.3	83	1091	2077
Body K, g	164.5	16.5	37	129.5	204.5
Body cell mass, kg	35.1	3.52	37	27.6	43.6
Calorie intake,[2] kcal/day	3343	317	83	2759	4380
kcal/kg body weight	46.4	4.8	83	35.5	57.4

[1] Basal metabolic rate.
[2] Calculated value.

both diets is given in table 2. Supplements of folic acid, vitamin E, zinc and magnesium were not included in the experimental diet. Even without specific supplementation, it seems unlikely that the healthy well-nourished subjects would have been significantly depleted during the relatively short experimental period. A customary fluid intake was determined for each individual, and then controlled at this level throughout the study. Since the urinary data (mg N per kg or g N per gram creatinine) and fecal N from the two groups were not significantly different when compared by either the paired t test or by analysis of variance ($P > 0.1$), they have been combined in the overall analysis. Hereafter, we refer to the diet for all subjects as a minimal protein diet.

TABLE 2

Composition of diets used for study of obligatory N losses in young men

Ingredient	Minimum protein Diet 1 [1]	Minimum protein Diet 2
Liquid formula [2]		
Homogenized oat meal, g	119	29
Whole, dried egg powder, g	—	13.4
Corn oil, g	115	140
Maltose, dextrins mixture,[3] g	250	200
Cellulose,[4] g	6	5
Pectin, g	1.5	—
Sodium chloride, g	1.0	1.0
Vanilla, g	10	10
Lemon juice, g	24	20
$Ca_{10}(OH)_2(PO_4)_6$, g	1.9	1.9
K_2HPO_4, g	5.7	5.7
Water, ml	400	450
Nonprotein calorie sources [5]		
Carbonated beverage	variable	
Sucrose soft drink	variable	
Cornstarch dessert	variable	
Protein-free cookies	variable	
Vitamin supplement [6]		
Mineral supplement [7]		

[1] A similar diet has previously been referred to as a "protein-free" diet (12).

[2] Subjects fed diet 1 were all given 934 g of the liquid formula per day. Subjects given diet 2 received 872 g of the liquid formula per 70 kg body weight per day.

[3] Burroughs-Wellcome and Co., Tuckahoe 7, New York. Approximate composition: 24% maltose, 76% dextrins.

[4] Avicel, Microcrystalline cellulose. Purchased from FMC Corporation, Newark, Del.

[5] Composition similar to those described previously (31). Intake varied among subjects, but was constant for any one subject.

[6] Unicap multivitamin supplement, The Upjohn Co., Kalamazoo, Mich. Each capsule contains: vitamin A, 5000 USP units; vitamin D, 400 USP units; (mg) thiamin-hydrochloride, 2.5; riboflavin, 2.5; ascorbic acid, 50; niacinamide, 20; pyridoxine hydrochloride, 0.5; calcium pantothenate, 5; and cobalamin, 2 μg; (as supplied by manufacturer).

[7] Ferrous sulfate capsules supplying 14 mg Fe per day. Donated by Smith, Kline and French Laboratories, Philadelphia, Pa. The spray-dried, pasteurized whole egg powder was donated by the British Egg Marketing Board, London; the oatmeal (Buckeye Rolled Oats, homogenized) was donated by the Quaker Oats Co., Barrington, Ill. The subjects were also allowed to chew 1 package per day of Trident sugarless gum (kindly donated by American Chicle Co., Long Island, N. Y.).

Methods and experimental design. The first 39 of these 83 subjects consumed the experimental diet for 16 days. Since satisfactory stabilization of urinary N excretion began as early as day 3 and never later than day 8, the duration of the study for the other 44 individuals was shortened to 14 days and conclusions were based on the 5-day period from day 10 through day 14. Complete 24-hour urine collections were obtained for each day of the study but were generally not analyzed for the first 3 days.

Fecal samples were pooled for 4- or 8-day periods and identified by the use of autoclaved carmine or brilliant blue markers (17). For 37 subjects, randomly selected from the total, BCM was estimated by measurement of whole body K (12), on 2 consecutive days at the beginning and again on the last 2 days of the study, and the average values were used.

Basal metabolism was determined by indirect calorimetry, with a Benedict-Roth respirometer, at the beginning and end of the experimental period. Subjects spent the night in the MIT Clinical Research Center so that BMR could be measured the following morning. Nitrogen and creatinine were determined in urine and N alone in feces and diet aliquots as previously described (12).

RESULTS

Figure 1 shows the change in urinary N output with respect to time for the 83 subjects. A stable level of urinary N excretion was achieved between days 3 and 8 for all individuals. The daily urinary N and creatinine excretions for days 10 through 14 (table 3) showed no statistically significant differences ($P > 0.5$) and therefore, the N excretion for each subject during this 5-day period was used for further evaluation of obligatory urinary N loss. These values are summarized in table 4. Also summarized in this table are the urinary, fecal and the sum of the urinary

TABLE 3

Analysis of variance of urinary N and creatinine excretion of 83 young men during days 10 to 14 on a minimal protein diet

Parameter	Day of experiment					Average days 10–14	SD between individ.	SD between days	SD "error"
	10	11	12	13	14				
Body weight, kg	72.8	72.7	72.5	72.5	72.5	72.6	9.07	0.13	0.39
Creatinine, g/day	1.74	1.73	1.73	1.73	1.67	1.72	0.18	—[1]	0.24
Urinary N									
g N/day	2.79	2.66	2.70	2.70	2.65	2.69	0.45	—[1]	0.43
mg N/kg body weight	38.3	36.4	37.4	37.3	36.6	37.2	4.8	—[1]	5.9
g N/g creatinine	1.62	1.54	1.56	1.57	1.59	1.58	0.19	—[1]	0.25
mg N/basal kcal	1.84	1.75	1.78	1.78	1.74	1.77	0.29	—[1]	0.27

[1] Not significantly different from zero ($P < 0.01$).

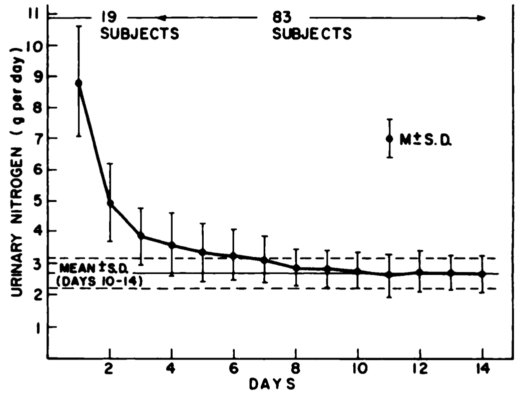

Fig. 1 Daily urinary nitrogen excretion of 83 young men given a minimal protein diet for 14 days. Values for the first 3 days are based on 19 subjects and subsequent values for 83 subjects.

and fecal N outputs, expressed in a number of ways.

Urinary N output was 2.69 ± 0.48 g, the variance being 18% of the mean. The coefficient of variance was essentially the same when urinary N was expressed per basal kilocalorie and slightly smaller when urinary N excretion was expressed either per gram of creatinine output or per kilogram of body weight. The cumulative distribution of urinary N per body weight (mg N/kg) is shown on a normal probability plot in figure 2. These data indicate that this parameter of N metabolism was normally distributed.

Fecal N excretion amounted to 0.63 ± 0.15 g per day, with a variance of 24% of the mean. The variance was similar for the other expressions of obligatory fecal N loss (table 4). For total N excretion, the mean daily output during days 10 to 14 was 3.33 ± 0.54 g per day or 46 ± 6 mg/kilogram body weight. The coefficient of variance for the combined urinary and fecal N loss per day was 16% and per kilogram body weight per day it was 13%.

The correlations between the obligatory N losses and body weight, BMR and creatinine excretion are summarized in table 5. Obligatory urinary excretion was significantly correlated ($P < 0.01$) with body weight ($r = 0.56$), creatinine ($r = 0.61$) and BMR ($r = 0.49$); fecal N correlated with body weight ($r = 0.35$) and creatinine output ($r = 0.36$).

TABLE 4

Mean obligatory urinary and fecal N losses of 83 young men receiving a minimal protein diet for 14 days

Parameter	Mean	SD	Coefficient of variance
			%
Urine [1]			
g N/day	2.69	0.484	18
g N/g creatinine	1.58	0.221	14
mg N/kg body wt	37.2	5.50	15
mg N/kg $^{0.75}$	10.85	1.59	15
mg N/basal kcal	1.77	0.297	17
Feces [2]			
g N/day	0.633	0.154	24
mg N/kg body wt	8.76	2.05	23
mg N/kg $^{0.75}$	2.55	0.587	23
mg N/basal kcal	0.418	0.105	25
Total excretion			
g N/day	3.33	0.538	16
g N/g creatinine	1.94	0.235	12
mg N/kg body wt	46.0	5.96	13
mg N/kg $^{0.75}$	13.4	1.70	13
mg N/basal kcal	2.19	0.339	16

[1] Values for days 10 through 14 of the experimental period.
[2] Values based on a pooled sample for the last 8 days of the period.

TABLE 5

Correlations between selected variables for 83 young men during days 10 to 14 of a minimal protein diet

	BMR	Creat.	U_N	F_N	T_N [1]
Body weight	0.64	0.75	0.56	0.35	0.60
BMR		0.62	0.49	0.26 [2]	0.52
Creatinine (creat.)			0.61	0.36	0.65
Urinary N (U_N)				0.21 [3]	0.56
Fecal N (F_N)					0.47

[1] $U_N + F_N$.
[2] Not significant at 1% level.
[3] Not significant at 5% level.

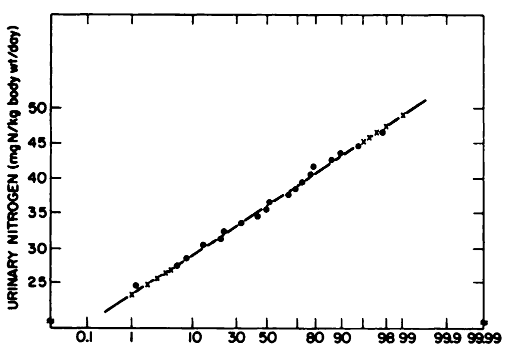

Fig. 2 A normal probability plot of obligatory urinary nitrogen excretion (mg N/kg body weight) for 83 young adult male subjects. The observed values (●) were derived from days 10 to 14 of the experimental period. Estimates of five highest and lowest percentiles are also indicated (x).

Although the dietary caloric intake, as calculated from a diet history, was sufficient to meet the individual's daily energy expenditure, the subjects lost an average of 0.9 kg body weight ($P < 0.01$) during the 2-week experimental period (table 6). This weight loss was paralleled by a mean decrease of 4 g ($P < 0.01$) in total body K, as determined by the total body ^{40}K changes.[4] This corresponds to the expected weight loss, if the observed K loss was due only to a change in BCM (18). This correspondence between the estimated and actual loss of BCM further supports the theory that the observed weight loss was due to the grossly inadequate level of pro-

[4] The decrease in body K was evaluated by paired *t* test and found to be statistically significant. Under our conditions the reliability in the ^{40}K estimate is usually within ±3 to 4%.

TABLE 6

BMR, total body potassium and body weight for 83 young men at the beginning and end of 14 days on minimal protein diets

Parameter	Initial [1]		Final		Change		Number of subjects
	Mean	SD	Mean	SD	Mean	SD	
BMR, kcal/day	1536	247	1533	227	−3	160	83
Total body K, g	165	16.5	161	15.1	−4.0 [2]	6.0	37 [3]
Body wt, kg	73.4	9.5	72.5	9.0	−0.9 [2]	1.0	83

[1] Data shown are for day on which BMR measurement was performed.
[2] Significantly different ($P < 0.01$) from zero.
[3] Twenty-one subjects received minimum protein diet 1, and 16 received minimum protein diet 2. Their body weights were 72 ± 9 kg and 71 ± 8 kg, respectively, at the time of the first ^{40}K measurement.

tein intake and not the result of an insufficient caloric intake. BMR did not change during the experimental period (table 6).

Figure 2 confirms the normal distribution for the obligatory urinary N loss in young adult male subjects, and table 3 indicates that the daily variation of each individual is similar to the variation in obligatory urinary N loss for the population as a whole. This raised the question of how characteristic the mean obligatory urinary N loss is for an individual over time. To help answer it, we located four of the subjects for restudy after a 3-year interval. The comparative values obtained during the 1966-67 study and 3 years later are summarized in table 7.

Two of the subjects had gained approximately 5 and 7 kg of body weight and this was reflected in significantly ($P < 0.05$) higher creatinine output, but not a higher BCM as estimated by ^{40}K measurement. In each case, the mean urinary N losses per kilogram body weight were not significantly ($P > 0.05$) different for the two periods; however, the individual who gained the most weight during the 3-year interval showed the largest difference. Although between the two periods the mean values of fecal N losses varied more than those for urinary N loss, we consider this due mainly to problems inherent in the use of fecal markers and in the sampling of feces rather than to actual biological variations.

DISCUSSION

Previous studies (3–14) have presented data for relatively few individuals, usually under the confining and protective conditions of the metabolic ward. In the present study the number of individuals is large and the subjects were exposed to the ordinary activities and stresses of everyday living. The only major restriction on the subjects other than the experimental diet was an obligation to refrain from competitive athletics or other unusual activities. The results should, therefore, be applicable to an ordinary healthy population and may also reflect variation due to stress from clinically unrecognized episodes of anxiety, frustration or infection. Two individuals who developed clinically evident and moderately severe upper respiratory infections did show a significant increase in urinary N excretion during the acute episode and were excluded from the present tabulations.

Our own previously published data (12) on obligatory urinary N losses from 11 individuals living in a metabolic ward with no academic or other responsibilities at the time gave a coefficient of variation of N excretion per kilogram body weight of 10% compared with 15% in the present study. The data obtained on 13 similar subjects by Calloway and Margen (13) give almost identical mean values and coefficients of variation for obligatory N losses as observed with the present 83 subjects.

Our data on whole body K gave an estimate of BCM, and the methods of underwater weighing and $^{3}H_2O$ dilution were used by Calloway and Margen to measure lean body mass. These two indicators of body composition are closely related and in both studies they were found to be correlated with N excretion ($P < 0.05$ in the present study). However, the latter authors, with a small sample and narrow range of body weight, found no significant

TABLE 7

Obligatory urinary and fecal N losses in four subjects given a minimal protein diet at 3-year intervals [1]

Parameter	Subject no.							
	14		54		57		68	
	1966	1969	1966	1969	1966	1969	1966	1969
Body weight, kg [2]	63.8 ± 0.13	70.7 ± 0.22 **	57.7 ± 0.23	62.5 ± 0.60 **	69.7 ± 0.13	68.3 ± 0.45 **	65.4 ± 0.24	66.9 ± 0.17 **
BCM, kg	34.5	34.9	29.2	28.5	39.4	38.4	—	—
BMR, kcal/day	1598	—	1226	—	1685	—	1397	1447
Creatinine, g/day [2]	1.52 ± 0.17	1.90 ± 0.09 *	1.34 ± 0.18	1.53 ± 0.06 *	1.85 ± 0.07	2.10 ± 0.28	1.63 ± 0.03	1.51 ± 0.38
Calorie intake,								
kcal/day	3531	3239	2759	2891	3189	3176	3321	3332
Urinary N [2]								
g/day	2.42 ± 0.52	3.24 ± 0.28 **	2.27 ± 0.13	2.27 ± 0.42	3.26 ± 0.24	3.27 ± 0.39	2.21 ± 0.27	2.47 ± 0.37
mg/kg body weight	38 ± 8	46 ± 4	39 ± 2	36 ± 7	47 ± 4	48 ± 6	34 ± 4	37 ± 6
g/g creatinine	1.6 ± 0.4	1.7 ± 0.1	1.7 ± 0.2	1.4 ± 0.3	1.8 ± 0.2	1.6 ± 0.2	1.4 ± 0.2	1.7 ± 0.5
Fecal N [3]								
mg/day	563	723	615	611	383	505	453	800
mg/kg body weight	8.8	10.2	10.7	9.8	5.5	7.4	6.9	12.0
Total N								
mg/kg body weight	46.7	56.0	51.6	46.1	52.2	55.3	40.7	49.0

[1] Differences between 1966 and 1969 were not significant except as noted: * significant difference ($P < 0.05$); ** highly significant difference ($P < 0.01$).
[2] Based on 5-day average value for days 10 to 14. Values are mean ± SD.
[3] Based on a pooled sample for the final 8 days of the period.

correlation between obligatory urinary N loss and total body weight.

In the present study, with a relatively large sample and a somewhat greater body weight range than for Calloway and Margen's subjects (13), the correlation coefficient for urinary nitrogen on body weight was statistically significant but small and relatively unimportant in explaining the variance in obligatory N loss of young adults. Clearly, within the range of age, body composition and body size characteristic of studies with healthy male university students, metabolic differences are more important than size differences in accounting for variation in obligatory N losses and protein requirements. For our population, the variation in body weight accounted for about 30% of the variation in urinary N excretion. When we expressed obligatory urinary N on a body weight basis, the coefficient of variation was reduced from 18 to 15%.

Mean obligatory urinary N output per basal kilocalorie was 1.8 (mg N/kcal) in the present study and 1.4 in that of Calloway and Margen (13). Previous workers (4, 8, 9) have also reported values for obligatory urinary N loss which are within this range, and all below the 2 mg N per basal kilocalorie assumed by the 1965 FAO/WHO Committee in their estimation of obligatory N losses in adult man (1). Furthermore, the coefficient of variation is greater when urinary N loss is expressed in this manner than on a body weight basis. This is related, in part, to the greater difficulties involved in assessing BMR than body weight. In addition, body weight was measured daily whereas BMR was measured only twice, at the beginning and at the end of the study. These results do not support the use of BMR as the reference base for describing obligatory urinary N losses in adult man. Since Fomon et al. (19) report a value of 0.6 to 0.8 mg of N per basal kilocalorie for infants, and 0.9 to 1.4 for 3- to 4-year-old children, it would further appear that extrapolation of adult values for urinary N per basal kilocalorie to children is wholly unjustified.

The mean obligatory fecal N loss of 9 mg N per kilogram body weight measured in the present study is not only less than half of that assumed by the 1965 FAO/WHO Committee on the basis of the then available data, but is also less than the 11 to 14 mg N value reported by others[5] (6, 7, 13). This figure can be assumed to vary with the nature of the experimental diet, with pathological changes in gastrointestinal function resulting from chronic malnutrition, and with acute or chronic infection including the presence of intestinal parasites. A possible explanation for the lower value observed in our study compared with studies with similar subjects also free of disease and malnutrition is the relatively low crude fiber content (less than 8 g crude fiber per day) of our experimental diet. This explanation does not appear to account for the difference between our results and those of Calloway and Margen (13), because their diet was also low in crude fiber content. Obligatory fecal N losses may be expected to be higher in most populations of developing countries where morphologic and functional changes in the gastrointestinal tract are common (20).

The present study was conducted primarily to provide a more reliable estimate of the mean and variation of obligatory urinary and fecal N losses in healthy young adult men. It was undertaken specifically because these losses formed the basis of the factorial approach used by the 1965 FAO/WHO Committee (1) and more recently by the U. S. Food and Nutrition Board (2) to assess the minimum physiological requirement for N in man. An important question, unanswered by the present study, is whether these losses represent the minimum physiological needs for total N and the individual essential amino acids.

The use of obligatory N loss has been criticized by Holmes (21) as a basis for estimating the protein requirements of man because the data of Deuel et al. (5) indicate that urinary N output is not stabilized during a 30-day protein-free period. For their single subject, it was clearly impossible to select an acceptable figure for obligatory urinary N loss, but his energy intake was grossly inadequate. The pres-

[5] Inoue, G., Y. Fujita and Y. Niiyama 1971 Studies on protein requirements with special reference to the effect of excess calories during adaptation of young men to low protein diets. I. Requirements of rice protein with excess and adequate calorie intakes (submitted for publication).

ent studies rule out this particular objection by Holmes (21) by showing that a stable rate of urinary N output is achieved in 3 to 8 days if an adequate energy intake is provided.

A more important reservation is the extent to which total obligatory N loss, as well as its variability among individuals, predicts minimum physiological needs for high quality protein in adult subjects. The variability in the total obligatory N loss observed with our 83 subjects is substantially lower than the range of variation in the requirements reported for the individual amino acids (22, 23) or for maintenance of N balance (13) in adults.

The results of Hegsted et al. (24) and of Bricker et al. (10) have been used to support the view that total obligatory N losses are equivalent to the minimum physiological need for dietary nitrogen. Miller and Payne (25) believe that estimates of minimum N needs based on the factorial method are adequate for maintaining N balance over short periods and good health over long periods. It is known, however, that body protein metabolism is sensitive to changes in amino acid and protein intake (cf. 26). When the dietary amino acid supply is inadequate, the endogenous amino acids, released during tissue protein breakdown, are more extensively reutilized (27, 28) for protein synthesis, and their rate of catabolism is reduced (29, 30).

The net effect of these metabolic changes is a relative conservation of endogenous amino acids when protein intake is significantly suboptimal. Therefore, if these metabolic changes lead to a more effective conservation and utilization of amino acids at a very low intake of N as compared with an intake approaching that sufficient to maintain N balance, it follows that the summated obligatory N losses would underestimate the actual minimum physiological N needs. Calloway and Margen (13) and Inoue et al.[6] have recently reported that the minimum amount of egg protein N required to maintain N balance in adult male subjects is significantly greater than that predicted from the summated obligatory N loss. We have completed studies which support this general conclusion.

It is pertinent to ask whether the results obtained with university students of Caucasian origin apply equally to population groups of differing racial origins and nutritional backgrounds. The data of Huang et al. (32) suggest that obligatory urinary N losses of healthy university students of Oriental origin in either the U. S. or Taiwan tend to be significantly less than those found in the present study.

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LITERATURE CITED

1. FAO/WHO 1965 Protein Requirements Report of Joint FAO/WHO Expert Group. WHO Tech. Report Series no. 301.
2. National Research Council Food and Nutrition Board 1968 Recommended Dietary Allowances, publ. 1964. National Academy of Sciences, Washington, D. C.
3. Martin, C. J., and R. Robison 1922 The minimum nitrogen expenditure of man and the biological value of various proteins for human nutrition. Biochem. J. *16:* 407.
4. Smith, M. 1923 The minimum endogenous nitrogen metabolism. J. Biol. Chem. *68:* 15.
5. Deuel, H. J., J. Sandiford, K. Sandiford and W. M. Boothby 1928 A study of nitrogen minimum. The effect of sixty-three-days of a protein-free diet on the nitrogen partition products in the urine and on the heat of production. J. Biol. Chem. *76:* 391.
6. Mueller, A. J., and W. M. Cox 1947 Comparative nutritive value of casein and lactalbumin for man. J. Nutr. *34:* 285.
7. Hawley, E. E., J. R. Murlin, E. S. Nasset and T. A. Szymanski 1948 Biological values of six partially-purified proteins. J. Nutr. *36:* 153.
8. Kofrányi, E. 1957 Zur Bestimmung der biologischen Wertigkeit von Nahrungsproteinen. III. Der Vergleich der Wertigkeiten von Milcheiweiss und Roggeneiweiss. Hoppe-Seyler's Z. *309:* 253.
9. Murlin, J. R., L. E. Edwards, E. E. Hawley and L. C. Clark 1946 Biological values of proteins in relation to the essential amino acids which they contain. I. The endogenous nitrogen of man. J. Nutr. *31:* 533.
10. Bricker, M. L., R. F. Shively, J. M. Smith, H. H. Mitchell and T. S. Hamilton 1949 The protein requirements of college women on high cereal diets with observations on the

[6] See footnote 5.

adequacy of short balance periods. J. Nutr. *37:* 163.
11. Bricker, M. L., and J. M. Smith 1951 A study of the endogenous nitrogen output of college women with particular reference to the use of the creatinine output in the calculation of the biological values of the protein of egg and sunflower seed flour. J. Nutr. *44:* 553.
12. Young, V. R., and N. S. Scrimshaw 1968 Endogenous nitrogen metabolism and plasma free amino acids in young adults given a "protein-free" diet. Brit. J. Nutr. *22:* 9.
13. Calloway, D. H., and S. Margen 1971 Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human protein requirement. J. Nutr. *101:* 205.
14. Gopalan, C., and B. S. Narasinga Rao 1966 Effect of protein depletion on urinary nitrogen excretion in undernourished subjects. J. Nutr. *90:* 213.
15. Smuts, D. B. 1935 The relation between the basal metabolism with particular reference to the estimation of the maintenance requirement of protein. J. Nutr. *9:* 403.
16. Shock, N. W., D. M. Watkin, M. J. Yiengst, A. H. Norris, G. W. Gaffney, R. J. Gregerman and J. A. Falzone 1963 Age differences in the water content of the body as related to basal oxygen consumption in males. J. Gerontol. *18:* 1.
17. Lutwak, L., and B. T. Burton 1964 Fecal dye markers in metabolic balance studies. The use of brilliant blue and methylcellulose for accurate separation of stool periods. Amer. J. Clin. Nutr. *14:* 109.
18. Moore, F. D., K. H. Olesen, J. D. McMurray, H. V. Parker, M. R. Ball and C. M. Boyden 1963 The Body Cell Mass and its Supporting Environment. W. B. Saunders Co., Philadelphia.
19. Fomon, S. J., E. M. DeMaeyer and G. M. Owen 1965 Urinary and fecal excretion of endogenous nitrogen by infants and children. J. Nutr. *85:* 235.
20. Tripathy, K., E. Duque, O. Bolanos, H. Lotero and L. G. Mayoral 1971 Malabsorption syndrome in ascariasis. Presented at a workshop on Malabsorption and Malnutrition, April 29–30, National Academy of Sciences–National Research Council. Amer. J. Clin. Nutr. 25: 1276.
21. Holmes, E. G. 1965 In: World Review of Nutrition and Dietetics, vol. 5, ed., G. H. Bourne. S. Karger, Basel/New York, pp. 237–274.
22. Rose, W. C. 1957 The amino acid requirements of adult man. Nutr. Abstr. Rev. *27:* 631.
23. Leverton, R. M., J. Ellison, N. Johnson, J. Pazur, F. Schmidt and D. Geschwender 1956 The quantitative amino acid requirements of young women. V. Leucine. J. Nutr. *58:* 355.
24. Hegsted, D. M., A. G. Tsongas, D. B. Abbott and F. J. Stare 1946 Protein requirements of adults J. Lab. Clin. Med. *31:* 261.
25. Miller, D. S., and P. R. Payne 1969 Assessment of protein requirements by nitrogen balance. Proc. Nutr. Soc. (Engl.) *28:* 225.
26. Waterlow, J. C. 1968 Observations on the mechanism of adaptation to low protein intakes. Lancet *2:* 1091.
27. Stephen, J. M. L., and J. C. Waterlow 1966 Use of carbon-14-labelled arginine to measure the catabolic rate of serum and liver proteins and the extent of recycling. Nature *211:* 978.
28. Said, A. K., and D. M. Hegsted 1970 Response of adult rats to low dietary levels of essential amino acids. J. Nutr. *100:* 1363.
29. Neale, R. J. 1971 Adaptation of amino-acid metabolism in protein-depleted rats. Nature *231:* 117.
30. McFarlane, I. G., and C. von Holt 1969 Metabolism of amino acids in protein–calorie-deficient rats. Biochem. J. *111:* 557.
31. Young, V. R., M. A. Hussein, E. Murray and N. S. Scrimshaw 1971 Plasma tryptophan response curve and its relation to tryptophan requirements in young adult men. J. Nutr. *101:* 45.
32. Huang, P. C., H. E. Chong and W. M. Rand 1972 Obligatory urinary and fecal nitrogen losses in young Chinese men. J. Nutr. *102:* 1605–1614.