Endogenous nitrogen metabolism and plasma free amino acids in young adults given a 'protein-free' diet*

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(*Received* 7 *February* 1967—*Accepted* 25 *July* 1967)

1. Variation in endogenous nitrogen metabolism was determined by giving eleven healthy men, aged 17–22, a diet supplying daily only 6 mg N/kg body-weight. Eight subjects were given the diet for 7–10 days and three other subjects were given it for 16 days.

2. Body cell mass (BCM) was calculated from whole-body ^{40}K in ten subjects and basal metabolism was determined in seven subjects during the 'protein-free' period. Urine was analysed daily for N and creatinine, and faecal N was measured in pooled samples. Plasma free amino acids, serum albumin and protein were measured in preprandial morning blood samples at the beginning and end of the study.

3. BCM did not change during the 'protein-free' period and accounted for 48% of the total body-weight. Basal calorie expenditure amounted to 48·5 kcal/kg BCM per day.

4. Mean daily endogenous urinary N excretion in the eight subjects given the 'protein-free' diet for 7–10 days was 36·6 ± 3·0 mg N/kg body-weight, 79·4 ± 4·4 mg N/kg BCM and 1·6 ± 0·2 mg N/basal kcal. Endogenous faecal N excretion was 9·9 ± 1·1 mg N/kg body-weight and accounted for 20% of the total endogenous loss. Results obtained with three other subjects given the diet for 16 days were similar.

5. Plasma essential amino acids were reduced, glutamic acid, alanine and glycine increased, and the ratio of essential to non-essential amino acids decreased after 7 or 10 days of 'protein-free' diet.

6. The loss of endogenous N per basal kcal and of faecal N per kg body-weight was lower than the values assumed in the factorial approach to protein requirements by the FAO/WHO (1965) Expert Group on Protein Requirements.

The two major components of man's protein requirements are: (1) the amount and relative proportions of essential amino acids, and (2) the quantity of nitrogen required for the synthesis of non-essential amino acids and other N-containing compounds. The requirements for essential amino acids have been determined in men and women having relatively high intakes of total N (Rose, 1957; Leverton, Ellison, Johnson, Pazur, Schmidt & Geschwender, 1956; Swendseid & Dunn, 1956), and the results suggest wide individual variation in the requirements for certain of the essential amino acids.

The need for lysine and the branched-chain amino acids varied among subjects by as much as two- to three-fold (Rose, 1957; Leverton *et al.* 1956). A number of factors may account for this finding. The efficiency of absorption and subsequent utilization of the amino acids may vary (Scrimshaw, 1962–3). Studies in both experimental animals (Harper, 1964) and human subjects (Scrimshaw, Bressani, Béhar & Viteri, 1958; Clark, Myers, Goyal & Rinehart, 1966) show clearly that the requirement for a particular amino acid is influenced by the relative proportions of other

* This manuscript is contribution no. 1035 from the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass.

acids present in the diet. Furthermore, the utilization of the essential amino acids is influenced by the total amount of N in the diet (Kies, Shortridge & Reynolds, 1965; Tuttle, Swendseid, Mulcare, Griffith & Bassett, 1959; Clark, Kenney, Goodwin, Goyal & Mertz, 1963), which in turn may accentuate variability among subjects.

Recent investigations conducted in our laboratory (Scrimshaw, Young, Schwartz, Piché & Das, 1966; Huang, Young, Cholakos & Scrimshaw, 1966) have been concerned with determining the minimum proportion of essential amino acids to total dietary N necessary for adequate protein nutrition in young adult subjects. Using whole-egg protein, which presumably has an ideal balance of essential amino acids (FAO/WHO, 1965), as the major protein component of the diet, we have noted a wide variation in the metabolic response of subjects to an isonitrogenous replacement of protein with a non-specific N source (Scrimshaw *et al.* 1966). Our results were obtained at relatively low levels of N intake, and we have examined the extent to which variations in endogenous N excretion may reflect the variability in amino acid requirements. The results obtained with eleven subjects given a 'protein-free' diet for 7–16 days form the basis of this report. Because of limited information about the excretion of endogenous N per unit of basal-energy expenditure in young adults (Murlin, Edwards, Hawley & Clark, 1946*a*; Deuel, Sandiford, Sandiford & Boothby, 1928; Smith, 1926; Bricker & Smith, 1951), we have, during these studies, also measured basal metabolism and examined the relationship between endogenous N excretion and body cell mass (BCM), calculated from whole-body ^{40}K content.

Although a number of studies have been concerned with the influence of low-protein diets on plasma amino acids (Swendseid, Tuttle, Figueroa, Mulcare, Clark & Massey, 1966), there appears to be a need for information on the influence of a 'protein-free' diet on plasma amino acid levels in healthy adults. This report also describes preprandial plasma amino acid levels in healthy, young adult males receiving 'protein-free' diets.

EXPERIMENTAL

The age, weight, height, protein intake before the experiment and calorie intake, calculated from food composition tables (Watt & Merril, 1963) and data supplied by the manufacturers of some of the dietary components, for the eleven subjects studied are given in Table 1. All were in good health as judged by medical histories and physical examinations at the beginning and the end of the experiments. The studies were conducted in the M.I.T. Clinical Research Centre, and the subjects were maintained under close nursing and medical supervision. They were required to continue their usual levels of physical activity throughout the study.

All subjects received adequate protein for at least a week before the 'protein-free' period (Table 1). The composition of the 'protein-free' diet is given in Table 2. It was found necessary to include a small amount of oatmeal in the fluid mixture to maintain a stable mixture of agreeable consistency. The diet supplied only 6 mg N/kg day and was therefore, designated as 'protein-free'. We have used this designation for the diet throughout the remainder of this paper.

Table 1. *Age, weight, height and calculated calorie intake of young men given a 'protein-free' diet for periods of 7–16 days*

Subject	Age (years)	Body-wt (kg)	Height (cm)	Calculated calorie intake (kcal/kg body-wt)	Protein intake before 'protein-free' period*: g/kg body-wt day	Protein intake before 'protein-free' period*: No. of days given
M.R.	18	75·8	183	35	0·8	7
I.J.	22	79·9	191	37	0·8	7
J.S.	18	67·5	175	40	0·8	7
G.C.	18	64·5	178	48	1·0	7
W.S.	19	64·8	168	40	1·0	7
J.G.	20	67·7	170	42	1·0	7
N.M.	17	72·7	174	38	0·8	14
B.R.	19	67·4	178	43	0·8	14
D.P.	18	66·9	178	43	0·8	14
R.W.	18	70·0	155	33	1·0	14
P.S.	22	86·8	188	36	1·3	30

* Protein was mostly of animal origin.

Table 2. *Composition of fluid 'protein-free' and maintenance protein diets used during the experimental periods*

	'Protein-free'		Maintenance	
	g/100 g	g/day*	g/100 g	g/day*
Whole dried egg†	—	—	4·4	49
Oatmeal‡	12·6	119	6·1	68
Dexin§	26·5	250	17·4	194
Maize oil	12·2	115	8·8	98
Glucose	—	—	0·4	4·5
Lemon juice	2·5	24	0·4	4·5
Avicel‖	0·5	4·7	0·2	2·2
Pectin	0·2	1·9	—	—
Vanilla	1·1	10	0·8	8·9
Lecithin	1·0	9	0·4	4·5
NaCl	0·1	1	0·1	1
K_2HPO_4	0·6	5·7	0·4	4·5
$Ca_{10}(OH)_2(PO_4)_6$	0·3	2·8	0·2	2·2
H_2O	42·4	400	60·5	675
Total	100	943	100	1116
Iron¶	—	—	—	—
Vitamins**	—	—	—	—
Tomato juice	—	—	—	100
Maize starch cookies and dessert	—	Variable††	—	Variable
Carbonated beverage	—	Variable	—	Variable

* Amount for a 70 kg subject.
† Spray-dried, pasteurized whole-egg powder(British Egg Marketing Board, London).
‡ Buckeye Rolled Oats, homogenized (Quaker Oats Company, Barrington, Illinois).
§ Approximate composition: dextrins 75%; maltose 24% (Burroughs Wellcome and Company, Tuckahoe, New York).
‖ Microcrystalline alpha cellulose (F.M.C. Corporation, Marcus Hook, Pennsylvania).
¶ One tablet supplying 14 mg Fe (Smith, Kline & French, Philadelphia, Pennsylvania).
** Unicap multivitamin capsule (Upjohn Company, Kalamazoo, Michigan).
†† Intake varied among subjects in order to adjust diet to individual calorie needs, but was constant for any one subject during the 'protein-free' or maintenance period.

Eight subjects were given the 'protein-free' diet for 7 or 10 days (Expt 1). The other three subjects were maintained on this diet for 16 days (Expt 2). Following 3 days on an *ad lib.* intake, seven of the subjects in Expt 1 were given a diet supplying a level of protein intake calculated to cover their minimum N requirement according to the FAO/WHO (1965) factorial approach for 14–16 days. This requirement was estimated from the sum of the urinary and faecal N losses during the latter part of the 'protein-free' period, plus a daily allowance of 15 mg N/kg for minimal integumental and sweat losses. An allowance of 20 mg N/kg body-weight, as proposed in the FAO/WHO report, was considered too high since these subjects were maintained in an air-conditioned environment and in view of the losses found by Mitchell & Hamilton (1949) and Consolazio, Le Matoush, Nelson, Isaac & Canham (1966). The composition of the maintenance diet is given in Table 2. Ninety per cent of the dietary protein at the calculated minimum requirement level was contributed by whole-egg protein with oatmeal and tomato juice each furnishing 5 %.

Body cell mass was calculated by determinination of total body ^{40}K burden, using the M.I.T. whole body counter. A value of 0·64 count/min per g K was used as the calibration factor for estimating total body K (Evans, 1964). The relationship between body K and N was assumed to be 3 m-equiv. K/g N; 1 g N is equivalent to 25 g of BCM. No correction was made for the extracellular K in view of the negligible error from this source (Moore, Olsen, McMurrey, Parker, Ball & Boyden, 1963).

Basal metabolism was determined by indirect calorimetry at the beginning and end of the experimental period for most subjects, using a Kofranyi–Michaelis respirometer. The O_2 and CO_2 content of expired gas was analysed by the Scholander micro-gas technique. The two determinations gave almost identical values, and the mean value is given in the results.

Complete 24 h collections of urine were made, and faecal samples were pooled. N determinations were made from urine, faeces and diet, and urinary creatinines were determined daily, all as previously described (Scrimshaw *et al.* 1966).

Blood for amino acid analysis was taken from the antecubital vein of subjects who had fasted for 10 h. The blood was analysed for free amino acids (Scrimshaw *et al.* 1966) and for serum total protein and albumin (Annino, 1964).

RESULTS

Table 3 summarizes results obtained for BCM, creatinine excretion and determinations of basal metabolic rate. BCM showed a tendency to decrease in most subjects following the 'protein-free' period. The average decrease of 2 % in BCM was within the 5 % sensitivity of the method. Expressed as a percentage, BCM accounted for 48 % of body-weight, and the calculated mean calorie expenditure of the BCM was 48·5 kcal/kg BCM day.

Urinary N excretion for eight subjects given the 'protein-free' diet for a 7- or 10-day period is shown in Table 4. For 7-day subjects the value for the last day is given since a steady-state N excretion was not apparently reached before that time. Because those given the 'protein-free' diet for 10 days showed a steady-state excretion after the 6th or 7th day, the mean value during the last 3 or 4 days is given. Expressed on a body-weight basis, the mean coefficient of variation (SD × 100/mean) of N excretion per kg in these eight subjects was 8·1 %. The mean daily value for seven subjects in which an estimation of BCM was made was 79·4 mg N/kg BCM with a mean coefficient of variation of 5·5 %. The relationship between urinary N excretion and basal metabolism is also shown in Table 4. The mean value for the eight subjects

Table 3. *Body cell mass (BCM), basal calorie expenditure and urinary creatinine excretion in young men given a 'protein-free' diet for 7–16 days*

Subject	BCM Before* (kg)	BCM After† (kg)	BCM % of body-wt	Basal calorie expenditure kcal/24 h	Basal calorie expenditure kcal/kg BCM day‡	Creatinine excretion (g/day)
N.M.	32·9	31·9	45‡	1779	54·9	1·62 ± 0·03§
B.R.	30·6	29·5	45	1580	50·8	1·64 ± 0·02
D.P.	32·0	31·6	48	1515	47·6	1·80 ± 0·02
R.W.	—‖	—	—	1556	—	1·51 ± 0·19
M.R.	37·4	37·2	49	1508	40·4	1·96 ± 0·13
I.J.	37·4	37·0	47	1668	44·8	1·94 ± 0·13
J.S.	32·1	32·5	48	1605	49·7	1·54 ± 0·23
P.S.	36·9	36·2	42	1876	51·3	1·87 ± 0·03
G.C.	33·9	33·9	53	—	—	1·72 ± 0·03
W.S.	31·5	29·8	47	—	—	1·61 ± 0·33
J.G.	36·0	36·4	53	—	—	1·71 ± 0·06
Mean	34·1	33·6	48	1636	48·5	1·72

* At beginning of 'protein-free' period.
† On last day of 'protein-free' period.
‡ Mean values for BCM used.
§ Mean values and standard deviations for last 4 days of 'protein-free' period.
‖ Not measured.

Table 4. *Expt* 1. *Endogenous urinary and faecal N excretion by young men given a 'protein-free' diet for 7–10 days*

Subject	Days on 'protein-free' diet	Urinary N/day g	Urinary N/day mg/kg body-wt	Urinary N/day mg/kg BCM*	Urinary N/day mg/basal kcal	Faecal N/day g	Faecal N/day mg/kg body-wt	Faecal N/day mg/kg BCM*	Faecal N/day % of total N excretion
N.M.	7	2·70†	37·1	84·6	1·5	0·63‡	8·7	19·7	18·9
B.R.	7	2·33	34·6	79·0	1·5	0·68	10·1	23·1	22·6
D.P.	7	2·32	34·6	73·4	1·5	0·62	9·3	19·6	21·1
R.W.	10§	2·70	38·6	—	1·7	0·56	8·0	—	17·2
M.R.	10	2·94	38·8	79·0	1·9	0·80	10·6	21·5	21·4
I.J.	10	3·05	38·1	82·4	1·8	0·73	9·1	19·7	19·3
J.S.	10	2·70	40·0	83·1	1·7	0·60	8·9	18·5	18·1
P.S.	10	2·69	31·0	74·1	1·4	0·63	7·3	17·4	19·0
Mean	—	2·68	36·6	79·4	1·6	0·66	9·0	19·9	19·7
SD	—	—	2·98	4·36	0·18	—	1·06	1·88	1·83

* Body cell mass.
† Value for 7th day of 'protein-free' period.
‡ Pooled faecal sample for last 3 or 4 days of 'protein-free' period.
§ Urinary values for subjects given the diet for 10 days are mean values for days 7–10.

was found to be 1·6 mg N/basal kcal, with a range of from 1·4 to 1·9. The overall coefficient of variation was approximately 11 %, which is slightly higher than the variability found for N excretion when expressed on the basis of body-weight.

Faecal N excretion for these subjects is summarized in Table 4. The mean daily rate of metabolic faecal N excretion was found to be 9·0 mg/kg body-weight with a coefficient of variation of 11·8 %. Faecal N appeared to account for 19·7 % of the total daily urinary plus faecal loss in these eight subjects during the last few days of the 'protein-free' period.

Table 5. *Expt 2. Mean urinary and faecal N excretion by young men given a 'protein-free' diet for 16 days*

Subject	Days of 'protein-free' diet	Urinary N/day g	Urinary N/day mg/kg body-wt	Urinary N/day mg/kg BCM*	Faecal N/day g	Faecal N/day mg/kg body-wt	Faecal N/day mg/kg BCM*	Faecal N/day % of total N excretion
G.C.	6–10	2·47	38·3	72·9	0·64	9·9	18·9	20·6
	11–13	2·26	35·0	66·7	0·85	13·2	25·1	27·3
	14–16	2·21	34·3	65·2	0·89	13·8	26·3	28·7
W.S.	6–10	2·25	34·7	75·5	0·51	7·9	17·1	18·5
	11–13	2·12	32·7	71·1	0·48	7·4	16·1	18·5
	14–16	2·82	43·5	94·6	0·65	10·0	21·8	18·7
J.G.	6–10	2·42	35·7	66·5	0·56	8·3	15·4	18·8
	11–13	2·41	35·6	66·2	0·39	5·8	10·7	13·9
	14–16	2·32	34·3	63·7	0·89	13·1	24·5	27·7
Mean	6–10	2·38	36·2	71·6	0·57	8·7	17·1	19·3
	11–13	2·26	34·4	68·0	0·57	8·8	17·3	19·9
	14–16	2·45	37·4	74·5	0·81	12·3	24·2	25·0

* Body cell mass.

Results for the three subjects given a 'protein-free' diet for 16 days in Expt 2 are summarized in Table 5. During days 6–10, 11–13, and 14–16, the mean urinary excretion values for the three subjects were 36·2, 34·4, and 37·4 mg N/kg body-weight per day, respectively, and these values compare well with those shown in Table 4 for subjects in Expt 1. Urinary N excretion on a BCM basis appeared to be slightly lower for these three subjects than for the seven subjects studied in Expt 1. The mean values of 71·6, 68·0 and 74·5 mg/kg BCM during days 6–10, 11–13, and 14–16, respectively, compare with 79·4 mg/kg BCM for the seven subjects studied during a shorter period of 'protein-free' diet.

Faecal N excretion in Expt 2 is summarized in Table 5. The results are in general agreement with those obtained in Expt 1 and shown in Table 4.

Following the 'protein-free' period, seven subjects were allowed 3 days on an *ad lib.* diet and then given protein at a level calculated to be close to their minimum N requirements for 14–16 days. Comparison of N excretion in subjects given a protein-maintenance diet with excretion in the same subjects given a 'protein-free' diet showed that the former level was significantly higher in all subjects following achievement of a relatively steady rate of N excretion. The mean rate of urinary N excretion was 59·1 mg/kg day for the protein diet compared with 37·4 mg/kg day for the 'protein-free' diet, a difference of 21·7 ± 1·97 (6 df). Mean faecal N excretion did not significantly increase after giving N at the calculated level of each subject's minimum requirement. The mean value was 9·7 mg/kg for the protein diet and 9·2 mg/kg for the 'protein-free' diet, a difference of 0·5 ± 0·6 (6 df).

Table 6. *Mean values for serum total protein, albumin and plasma free amino acids and mean differences with their standard errors after giving two groups of young men a 'protein-free' diet for 7–16 days*

	Group 1†		Group 2‡	
	Day 0	Difference at 7 or 10 days	Day 0	Difference at 16 days
Serum protein (g/100 ml)	7·1	−0·3 ± 0·08**	6·8	−0·2
Serum albumin (g/100 ml)	4·3	−0·1 ± 0·04*	4·0	+0·4
Essential amino acid§ (μmoles/l.):				
Threonine	233	− 16 ± 12	212	− 57
Valine	260	− 79 ± 9**	217	− 57
Cystine (half)	71	− 7 ± 7	66	0
Isoleucine	91	− 17 ± 4**	80	− 15
Leucine	166	− 26 ± 5**	139	− 23
Tyrosine	75	− 10 ± 3**	60	− 6
Phenylalanine	73	− 6 ± 3	62	− 2
Lysine	239	− 31 ± 8**	209	− 40
Total (*E*)	1208	−192 ± 35**	1045	−200
Non-essential amino acid (μmoles/l.):				
Aspartic	41	+18 ± 6*	41	− 7
Serine and glutamine	199	+9 ± 46	194	− 6
Glutamic	307	+102 ± 19**	228	+ 36
Citrulline	40	+5 ± 3	41	− 2
Glycine	319	+96 ± 22**	280	+ 86
Alanine	447	+329 ± 35**	315	+397
α-Aminobutyric	27	−15 ± 2**	35	− 22
Ornithine	76	−5 ± 4	72	− 5
Histidine	105	−12 ± 5*	98	− 7
Arginine	116	−6 ± 9	98	+ 9
Total (*N*)	1682	+521 ± 53**	1402	+480
Ratio (*E*:*N*)	0·72	−0·28 ± 0·03**	0·75	−0·29

Positive sign indicates increase; negative sign indicates decrease.
*, ** Difference significant by paired *t* test at 0·05 and 0·01 levels, respectively.
† Group 1: seven subjects from Expt 1 and three subjects from Expt 2.
‡ Group 2: mean values from three subjects in Expt 2.
§ Methionine peak was too low for accurate determination and was omitted from the results. Tryptophan was not determined.

As shown in Table 6, the concentrations of serum albumin and total protein were slightly, though significantly, decreased by 'protein-free' intake for 7–10 days. The mean plasma albumin level showed an increase on the 16th day in the three subjects studied during Expt 2. The basal (day 0) amino acid values refer to analysis of blood samples taken before the 'protein-free' regimen while all subjects (group 1) were receiving a diet containing 0·8–1·0 g protein/kg day. The concentrations of valine, isoleucine, leucine, tyrosine and lysine were significantly reduced ($P < 0·01$) 7 days after the 'protein-free' diet was started. Similar results were obtained for three subjects (group 2) after 16 days of giving the 'protein-free' diet. Threonine appeared to show a greater decrease after 16 days than during the first 10 days of the experiment. The total essential amino acids decreased significantly ($P < 0.01$) after giving the 'protein-free' diet for 7 or 10 days.

The plasma non-essential amino acids responded variably to a protein-free regimen. Aspartic acid, glutamic acid, glycine and alanine increased significantly after 7 or 10 days. α-Aminobutyric acid decreased to some 30–50% of the control values in these two experiments. The total non-essential amino acids increased ($P < 0.01$), and the ratio of essential to non-essential amino acids (ratio $E:N$) decreased ($P < 0.01$) significantly after 7–10 days on the 'protein-free' diet but showed no further change by day 16. The results obtained with three subjects after 16 days of giving the 'protein-free' diet were similar to those obtained after 10 days on the 'protein-free' diet.

DISCUSSION

Endogenous urinary N

On the basis of results for five animal species (mice, rats, guinea-pigs, rabbits, pigs), Smuts (1935) proposed a value of 2 mg N/basal kcal for the relationship between basal metabolic rate and endogenous urinary N. This value was used by the recent FAO/WHO Expert Group on Protein Requirements to estimate the protein requirement of man by the factorial approach (FAO/WHO, 1965). However, for the eight subjects studied in the present experiments, the relationship between urinary N and metabolic rate was found to be 1.6 ± 0.2 mg N/basal kcal, a value substantially lower than that used by the FAO/WHO Expert Group. On the basis of our limited results, endogenous urinary N excretion would be less than 2 mg N/basal kcal in approximately 98% of the adult population.

Our results are similar to the value of 1·48 mg N/basal kcal found by Murlin *et al.* (1946*a*) for five young men and to the value of 1·7 mg N/basal kcal found by Smith (1926) for a healthy young man after 23 days of eating a low-protein diet.

Recently, Gopalan & Narasinga Rao (1966), in their studies of endogenous N excretion in four undernourished subjects, found a mean value of 1·4 mg/basal kcal. Fomon, DeMaeyer & Owen (1965) calculated, for adult men, a value of 1·2 mg/basal kcal from the N balance results of Hawley, Murlin, Nasset & Szymanski (1948), and a value of 1·5 mg/basal kcal from the results of Mueller & Cox (1947). Using their own N balance results, Fomon *et al.* (1965) arrived at a value of only 0·6–0·8 mg N/basal kcal for infants aged 4–6 months and 0·9–1·4 mg N/basal kcal for 3- to 4-year-old children.

Judging from the results of Fomon *et al.* (1965) for children compared with the values for adults, urinary N excretion per kcal of basal metabolism apparently increases with age. Similar results have been recorded for rats by Ashworth (1935) and Ashworth & Cowgill (1938), who found that N excretion for weanlings was 1·0 mg/basal kcal compared with 1·5 mg/basal kcal for adults.

Urinary N excretion appeared to reach a relatively constant level after the 6th or 7th day of a 'protein-free' diet, and this level was maintained for the remaining 4 or 10 days of study. These results are in agreement with those obtained for both children and adult subjects. As would be expected, the previous level of protein intake influences the time at which a relatively constant rate of excretion is achieved. Gopalan & Narasinga Rao (1966) found that urinary N excretion reached a steady value within 2–3 days in undernourished adults.

The results obtained for man appear to differ from those reported for experimental animals. Allison & Wannemacher (1965) showed that urinary N excretion became relatively constant in rats and dogs only after a loss of about 25 % of body N. In contrast, a constant rate of excretion in an adult man is attained after approximately 5 % of body N has been lost (Munro, 1964). There may be, therefore, species differences in the metabolic response to a 'protein-free' diet.

The additional N above the level of urinary endogenous excretion which is lost during the first few days on a 'protein-free' diet has been taken to represent labile body protein. Martin & Robison (1922) developed a procedure for estimating an empirical rate constant (k) related to rate of urinary N excretion when subjects are given a 'protein-free' diet. For two adult males, they obtained a mean value for k of 0·35. Using their equation, Munro (1964) calculated an average value of 0·22 for rats and 0·18 for dogs. Although these values are highly tentative, they tend to support our view that man responds differently from the rat and dog to a 'protein-free' diet, if judged by the rate at which a relatively constant level of urinary N excretion is attained.

Basal urinary N excretion was found to be $36·6 \pm 3·0$ mg/kg body-weight per day in the eight subjects studied for a 7- or 10-day 'protein-free' period; similar values were obtained in three additional subjects during days 6–16 of a 16-day 'protein-free' period. These values are in reasonable agreement with those summarized by Fomon *et al.* (1965). The standard deviation of N excretion among these eleven subjects was about 10 % of the mean. Although this is substantially lower than the range of variation in the requirement for individual amino acids referred to earlier (Rose, 1957; Leverton *et al.* 1956; Swendseid & Dunn, 1956), it is identical with the variation in endogenous N loss assumed by the FAO/WHO Expert Group on Protein Requirements (FAO/WHO, 1965). In view of these findings, endogenous N loss does not reflect to any great extent the much greater variability in individual amino acid requirements, and other factors, either physiological or experimental, must play a more significant part in this respect. It should be emphasized, however, that these values were obtained from subjects studied within the sheltered environment of a hospital metabolic ward. It is possible that endogenous N excretion would show greater variability among subjects studied under the usual conditions of daily living.

Faecal N loss

Faecal N loss on a 'protein-free' diet has been shown to account for approximately 20 % of the combined urinary and faecal N loss, with mean daily values of 9·0 mg/kg body-weight in Expt 1 and 5·8–13·8 mg/kg body-weight during days 6–16 in Expt 2. Hawley *et al.* (1948) determined that faecal N loss accounted daily for 28 % of the total N loss and amounted to 11 mg/kg body-weight. Mueller & Cox (1947) found that it amounted to 13 mg/kg and 22 % of the total N loss during days 4–6 of a 'protein-free' diet; these results are in good agreement with ours. In children, metabolic faecal N loss accounts for approximately one-third of the total excretion (Fomon *et al.* 1965). The values found in the present study and those of the above workers are substantially lower than the 20 mg faecal N/kg body-weight allowed for by the FAO/WHO Expert Group (FAO/WHO, 1965) in assessing the protein requirements of man.

When subjects were given a level of N intake calculated to approximate to their minimum requirement, including 15 mg N/kg body-weight per day for cutaneous loss, urinary N excretion reached a constant level which averaged 58 % higher than that observed during the 'protein-free' period. Faecal N losses were not significantly higher than those observed during the 'protein-free' period.

In their studies of the biological value of egg protein, Murlin *et al.* (1946*a*) used a level of protein intake calculated by adding the faecal and urinary N losses measured 4 days after their subjects had been on a diet supplying less than 0·4 g N/day. These workers did not observe as great an increase in urinary N excretion during the period in which the minimum requirement of protein was given as was found in the present study. As a result, their calculated biological value for egg protein given at requirement levels was 88·6 (Murlin, Edwards, Hawley & Clark, 1946*b*) in contrast to a value of 65 found in our study. The most probable explanation is that the egg powder used in our study was damaged in processing or storage and did not have the expected biological value. Determination, by the method of Miller & Bender (1955), of the net protein utilization (NPU) for rats maintained in air-conditioned quarters at 24° revealed a value of 79 ± 2 for the dried whole-egg powder used in these experiments compared with 63 ± 2 for casein and values of 93–97 reported by Venkat Rao, Daniel, Joseph, Sankaran & Swaminathan (1964) for whole-egg powder. The present NPU results would preclude the use of these values for estimating obligatory N loss when young men are given a level of whole-egg protein calculated to meet maintenance needs.

Grande (1961) has estimated the mean calorie expenditure per kg of 'active tissue' to be 1·6 kcal/kg 'cells' h, or 38·4 kcal/kg 'cells' day. This value may be compared with those shown in Table 3 which vary between 40·4 and 54·9, with a mean value of 48·5 kcal/kg BCM day. However, Moore *et al.* (1963) suggest a range of 64·8–86·4 kcal/kg BCM day, substantially higher than the values found in the present experiments or in those of Grande (1961) and Grande, Anderson & Keys (1958). BCM was found to be approximately 48 % of body-weight in the present experiments, compared with the range of 35–45 % calculated by Moore *et al.* (1963) from total body exchangeable K, determined with ^{42}K.

Plasma amino acids

Of the free essential amino acids in the plasma, valine, isoleucine, leucine, tyrosine and lysine decreased significantly after 7 or 10 days of giving a 'protein-free' diet. The responsiveness of plasma valine in older men given a low-protein diet supplying 3·5 g N/day has recently been discussed by Swendseid *et al.* (1966). The branched-chain amino acids are reduced in children suffering from kwashiorkor and marasmus (Arroyave, Wilson, de Funes & Béhar, 1962; Holt & Snyderman, 1965). The present study shows that the levels of these amino acids in the plasma respond rapidly to acute protein deprivation in young adults.

The plasma $E:N$ ratio decreased after 10 days of 'protein-free' intake, but did not appear to decrease further by day 16 in Expt 2. Swendseid *et al.* (1966) have suggested that a low $E:N$ ratio might be taken as an indicator of some degree of protein depletion. Though the present studies and those of Swendseid *et al.* (1966) show that the ratio changes with adequacy of protein intake in healthy adults, the reliability of the ratio as an index of protein status requires further evaluation in view of the small, though statistically significant, change of serum albumin concentration during the 7 or 10 days after giving a 'protein-free' diet in the present experiments. It is relevant to record that Whitehead & Dean (1964) and McLaren, Kamel & Ayyoub (1965) have failed to demonstrate a correlation between the imbalance of essential to the non-essential amino acids in plasma and the clinical degree of protein malnutrition.

The authors acknowledge the kind aid of Dr Kolenkow of the Department of Physics, M.I.T., for arranging the use of the whole body counter and of Dr J. B. Das for his supervision of the subjects and basal metabolic rate measurements. We are indebted to M. L. Piché, Dr H. Zyas Byzan, F. Larin, and C. Squires for their technical assistance. The co-operation of the subjects who participated in these experiments is greatly appreciated.

This study was supported in part by USA National Institutes of Health, grant no. AM 06274 and utilized facilities of the M.I.T. Clinical Research Center financed by grant no. GM 70738.

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Printed in Great Britain