Nitrogen Balance Studies in Humans: Long-Term Effect of High Nitrogen Intake on Nitrogen Accretion[1]

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ABSTRACT Six healthy young adult male participants were confined to a metabolic ward for 105 days. Two nutritionally adequate purified diets providing 12 and 36 g of nitrogen per day were randomized in two metabolic periods of approximately 50 days each. The objective of this study was to verify whether or not positive nitrogen balance is a concomitant of increased nitrogen intake under the most rigorously controlled conditions, and if so, whether adaptation could occur if the experiment was conducted for sufficiently long periods of time. The mean nitrogen balance was slightly negative for most participants when fed the 12 g N diet. However, individual variability was so large that statistically all the participants can be considered in balance. In view of this, we agree with other investigators who have suggested that balance should be considered as an area which takes into account variabilities such as intake, output, and biological factors. On the 36 g N diet, all the participants exhibited strong positive balances, about 1.6 g/day, which were not as high as reported by other investigators but which persisted for as long as they were fed this diet. This positive balance could not seem to be explained on the basis of methodological errors or to any unmeasured nitrogen losses. There was no significant trend towards adaptation as claimed. J. Nutr. *109:* 363–377, 1979.

INDEXING KEY WORDS nitrogen balance · nitrogen retention · high nitrogen intakes · human nitrogen requirements

Attempts have been made using different approaches over the years to quantify the amount of nitrogen needed by man for growth and maintenance of health. Two physiological approaches were used by the 1973 FAO/WHO Ad Hoc Expert Committee on Energy and Protein Requirements for estimating nitrogen requirements for man: the factorial method and the nitrogen balance method (1). It is known, however, that the factorial method, among other shortcomings, underestimates requirements by about 30% (2). The nitrogen balance technique is a more logical approach than that of measuring nitrogen output on a nitrogen-free diet since, obviously, one of the most important features of a satisfactory protein intake for adults is that it must be capable of maintaining the subject in nitrogenous equilibrium (3). The normal, healthy matured adult male who is supposed to have ceased growing and has little or no provision for nitrogen storage should be able "to drift to equilibrium" whatever the nitrogen intake (4). This idea of drifting to equilibrium does not usually hold with high nitrogen intakes. Consequently, one of the criticisms of the nitrogen balance method is the unaccountable accretion of nitrogen in the body with high

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TABLE 1

Physical characteristics of the subjects

Subject	Age	Height	Initial body wt.
	yr	*cm*	*kg*
3601	26	170.5	74.2
3602	30	180.5	73.2
3603	28	173.5	73.0
3604	23	179.0	72.7
3605	25	183.5	73.3
3606	23	184.0	88.1

TABLE 2

Dietary design

Subject	Diet[1]	
	Metabolic period 1 (48 days)	Metabolic period 2 (57 days)
3601	A	B
3602	B	A
3603	A	B
3604	B	A
3605	A	B
3606	B	A

[1] Diet A: eggwhite protein (12 g N). Diet B: eggwhite protein (12 g N) + soy protein (24 g N) = 36 g N.

nitrogen intakes. The occurrence of this phenomenon—increased nitrogen retention with increased nitrogen intake—has received many explanations. Some of the reasons given are: (a) methodological errors (5); (b) slow physiological adjustments of the body to dietary nitrogen level changes (6); (c) true nitrogen accretion; and even (d) conversion of NH_3^+ to elemental nitrogen and the loss of the gaseous nitrogen through the breath (7).

The objective of this study was to verify

TABLE 3

Composition of 12 g N basic formula diet[1]

Main nitrogen source: Eggwhite protein (12 g N)	
Ingredient	Amount per day
	g/day
Egg albumin[2]	97.66
Dextrimaltose[3]	79.56
Cornstarch[4]	39.19
Sucrose[5]	44.22
Oil[6]	77.24
Calcium citrate ($Ca_3(C_6H_5)_7)_2 \cdot 4H_2O$)[7]	1.42
Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)[8]	3.88
Magnesium oxide (MgO)[8]	0.40
Potassium chloride (KCl)[8]	3.64
Potassium phosphate, monobasic (KH_2PO_4)[8]	2.75
Sodium chloride (NaCl)[8]	1.08
Biotin[9]	0.0002
Water, deionized	460.00
Mineral solution[10]	
Calcium citrate ($Ca_3(C_6H_5O_7) \cdot 4H_2O$)[7]	0.67
Phosphoric acid, conc. (H_3PO_4)[11]	1.83 (1.05 ml)
Hydrochloric acid, conc. (HCl)[11]	0.71 (0.60 ml)

Extra-energy formula and Vitamin and trace mineral supplements

Ingredient	Amount per day	
	Extra-energy formula[12]	
	g/100 g/day	*g/day*
Dextrimaltose[13]	16.0	35
Cornstarch[14]	12.8	28
Sucrose[15]	10.0	22
Oil[16]	5.9	13
Water, deionized	55.3	121

TABLE 3—(*Continued*)

Vitamin and trace mineral supplements				
				Total amount
Vitamins[17]				
Capsule C-3				
Thiamin	2.0			2.0 mg
Riboflavin	3.0			3.0 mg
Pyridoxine	5.0			5.0 mg
Vitamin B-12	2.0 μg			2.0 μg
d-Biotin	0.05			0.05 mg
Vitamin K-1	1.0			1.0 mg
Vitamin C (ascorbic acid)	55.0			55.0 mg
Capsule C-5				
Vitamin E (as d-α-tocopherol acetate)	35.0 IU			35.0 IU
Folic acid	0.5			0.5 mg
Niacinamide	20.0			20.0 mg
d-Calcium pantothenate	10.0			10.0 mg
Capsule C-6				
Vitamin A (as retinol acetate)	2,000.0 IU			2,000.0 IU
Ergocalciferol	200.0 IU			200.0 IU
Choline[18]				
Choline bitartrate	520.0 (tablet)			1,000.0 mg
Trace minerals[19]				
$FeSO_4 \cdot 7H_2O$	16.7	Fe^{++}		10.0 mg
$CuCl_2 \cdot 2H_2O$	1.79	Cu^{++}		2.0 mg
$ZnSO_4 \cdot 7H_2O$	21.9	Zn^{++}		15.0 mg
$MnSO_4 \cdot H_2O$	5.12	Mn^{++}		4.9 mg
$Na_2MoO_4 \cdot 2H_2O$	0.21	Mo^{+++}		0.24 mg
$Cr_2(SO_4)_3 \cdot 15H_2O$	1.07	Cr^{+++}		0.5 mg
$Na_2SeO_3 \cdot 10H_2O$	0.0162	Se^{++++}		11.0 mg
$AlK(SO_4) \cdot 12H_2O$	28.3	Al^{+++}		4.8 mg
KI	0.0653	I^-		0.15 mg
NaF	0.73	F^-		1.0 mg

[1] Diet started on day 9 of first period. [2] Pasteurized, spray-dried eggwhite solids. Seymour Foods, Inc., Topeka, Kansas 66601. [3] Matrin-42. Spray-dried corn syrup solids. Mead Johnson Labs., Evansville, Indiana 47721. [4] Buffalo 3401. CPC International, Englewood Cliffs, New Jersey 07632. [5] Pure cane granulated. C & H Sugar Co., San Francisco, California 94106. [6] Corn oil, Mazola. CPC International Inc., Industrial Division, Englewood Cliffs, New Jersey 07632. [7] MC/B. P.O. Box 7203, Los Angeles, California 90022. [8] Mallinckrodt Chemical Works, St. Louis, Missouri 63160. [9] Cal Biochem., San Diego, California 92112. [10] Supplemental mineral solution added; 8 ml of solution per day. [11] Allied Chemical, Morristown, New Jersey 07960. [12] One gram formula = 2 kcal. [13] Matrin-42. Spray-dried corn syrup solids. Mead Johnson Labs., Evansville, Indiana 47221. [14] Buffalo 3401. CPC International, Englewood Cliffs, New Jersey 07632. [15] Pure cane granulated. C & H Sugar Co., San Francisco, California 94106. [16] Corn oil, Mazola. CPC International Inc., Industrial Division, Englewood Cliffs, New Jersey 07632. [17] One each per day. Courtesy of Miles Laboratories, Elkhart, Indiana 46514. [18] Four per day. Wm. T. Thompson Company, Los Angeles, California 90745. [19] Three per day. Staynor Company, Berkeley, California 94703.

whether or not positive nitrogen balance is a concomitant of elevated nitrogen intake under the most rigorously controlled conditions and, if so, what the reasons might be and whether adaptation could occur if the experiment was conducted for sufficiently long periods of time. This first paper will focus mainly on the validity of the first three reasons given for the occurrence of this phenomenon. The second and third subsequent papers will verify whether the unaccountable nitrogen retention is due to evolution of elemental nitrogen through the breath or to shifts in body composition, respectively.

PROCEDURES

Subjects. Six healthy young adult men,[2] 23 to 30 years, with heights ranging from

[2] The experimental protocol was approved by the Committee for Protection of Human Subjects, University of California, Berkeley. The participants were informed of the purpose, nature and design of the experiment and any potential hazards involved before signing consent forms.

TABLE 4

Composition of 36 g N basic formula diet[1]

Main nitrogen source:	Eggwhite protein (12 g N) Soy protein (24 g N)
Ingredient	Amount per day
	g/day
Soybean protein isolate[2]	160.46
Egg albumin[3]	100.08
Dextrimaltose[4]	30.89
Cornstarch[5]	15.22
Sucrose[6]	44.22
Oil[7]	45.13
Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)[8]	2.30
Magnesium oxide (MgO)[8]	0.24
Potassium chloride (KCl)[8]	4.73
Biotin[9]	0.0002
Water, deionized	796.70
Extra-energy formula; Vitamin and trace mineral supplements[10]	

[1] Diet started on day 9 of the first period. [2] Promine F (91.5% protein). Central Soya, Procter and Gamble, Cincinnati, Ohio 45247. [3] Pasteurized, spray-dried eggwhite solids. Seymor Foods, Inc., Topeka, Kansas 66601. [4] Matrin-42. Spray-dried corn syrup solids. Mead Johnson Labs., Evansville, Indiana 47721. [5] Buffalo 3401. CPC International, Englewood Cliffs, New Jersey 07632. [6] Pure cane granulated. C & H Sugar Co., San Francisco, California 94106. [7] Corn oil, Mazola. CPC International, Englewood Cliffs, New Jersey 07632. [8] Mallinckrodt Chemical Works, St. Louis, Missouri 63160. [9] Cal Biochem., San Diego, California 92112. [10] See Table 3, footnote 12.

174 to 184 cm and weighing between 72 and 89 kg, volunteered to participate in the study (table 1). Before final admission into the study, all prospective candidates were interviewed, given a complete physical examination and were required to spend a whole day in the metabolic ward to familiarize themselves with the daily routines including the eating procedures. All the six participants except one who were finally selected stayed for the entire 105 days of the study; the one who left, 3601, departed at the end of day 81.

All the participants were housed in a metabolic ward, "The Penthouse," for the duration of the experiment under 24-hour continuously supervised nursing care (2). Fitness was maintained by the two 30-minute periods of walking on a treadmill at 3 mph up a 10% grade; the men were otherwise ambulatory but quite sedentary.

Experimental design. The experiment was divided into two consecutive metabolic periods, 48 and 57 days long, respectively (MP 1 and MP 2).

Diet. To minimize losses of intake, two nutritionally adequate purified liquid formula diets, containing two levels of nitrogen, 12 and 36 g/day, were fed during the two metabolic periods in a randomized fashion (table 2). The compositions of the basic diets, together with the extra-energy formula, vitamin and trace element supplements, are given in tables 3 and 4. One quarter of the daily formula allowance, of a milkshake consistency, was fed at 0830, 1230, 1630, and 2030 hours. For variety, weighed amounts of low-protein bread, low-protein rusks, protein-free margarine, fruit, decaffeinated coffee, and instant tea (table 5) were added to the basic formula diets at specific meal times during the study.

Initially, all the participants were placed on an energy intake of 40 kcal/kg body weight/day. An adjustment in energy intake was made when a ±0.75 kg weight change occurred, by an addition or subtraction of the necessary amount of the

TABLE 5

Additional diet items

Food item	Amount per day	When eaten[1]
	g/day	
Low-protein rusks[2]	50	B, S
Low-protein bread[3]	35	L
Protein-free margarine[4]	35	ad lib.
Fruit[5]	250	D
Decaffeinated coffee[6,7]	4.6	B, S
Instant tea[7,8]	1.0	L, D

[1] B = Breakfast (8:30 AM); L = Lunch (12:30 PM); D = Dinner (4:30 PM); S = Supper (8:30 PM); ad lib. = at subjects' discretion. [2] Aproten, manufactured in Italy by Carlo Erba S.p.A. (Milan), distributed by General Mills Chemicals, Inc., Minneapolis, Minnesota 55427. [3] Dietetic Paygel, General Mills Chemicals, Inc., Minneapolis, Minnesota 55427. [4] Kosher, unsalted, Manischewitz Co., Newark, New Jersey 07632. [5] Fruits for salad, sliced peaches, sliced pears, 8½ to 8¾ oz. pack in 3-day rotation. Courtesy of Del Monte, San Francisco 94106. [6] Sanka, Instant, General Foods Corporation, 250 North Street, White Plains, New York 10625. [7] Each beverage serving was brought up to 200 ml with deionized water. [8] Lipton Co., Inc., Englewood Cliffs, New Jersey 07632.

extra-energy formula. This extra-energy formula was designed to provide 2 kcal/g when hydrated, and the daily amount was provided in four equal meals. The adjustment of energy intake to keep body weight constant was only done, however, during metabolic period 1. That is, during MP 2, energy intake was kept constant in spite of any changes in body weight. This was done mainly to test whether the energy intake in MP 1 was sufficient for efficient utilization of the amount of nitrogen fed and to avoid any potential effects on nitrogen metabolism by the alterations in energy intakes (table 6).

All food items were accurately weighed to 0.1 g on an analytical balance prior to serving. Initially, all participants were instructed how to consume each meal quantitatively. At each meal, a clean, smooth plastic placemat was placed under all the food for each participant and all food items were consumed with the men leaning over the placemat so that any spills could be quantitatively recovered and consumed. The participants were required to clean the tumblers with small rubber spatulas, rinse the tumbler twice or more with deionized water, and to drink the rinsings. Any spills which could not be recovered were estimated and subtracted from the nutrient intake for that day or a duplicate sample was fed.

Deionized water was used in all parts of the diet. The men were allowed deionized water ad libitum above a minimum for drinking but were required to measure and record all intakes using graduated beakers provided for this purpose.

Daily toothbrushing was permitted, but without any dentifrice and spitting. All rinses were swallowed.

Samples and measurements. The health of the participants was monitored constantly. Pulse and body temperature readings were taken twice daily.

A composite of an entire day's intake, with the exception of the extra-energy formula and flavorings which were analyzed separately, was analyzed for nitrogen by a modified micro-Kjeldahl procedure using a selenium catalyst (8). The sum of the nitrogen content of the composite together with the extra-energy formula supplement was used to calculate daily individual intakes.

All urine samples were collected on a 24-hour basis (0800 hours to 0800 hours the next day) with no preservative except refrigeration at −10° for the first 57 days of the study. From day 58 until the end of the study, 5 ml of 6 N HCl was added to

TABLE 6

Energy intake

Subject	Metabolic periods					
	Period 1 (days 1 to 48)			Period 2 (days 49 to 105)		
	Mean body wt.	Energy intake		Mean body wt.	Energy intake	
		Mean	Range		Mean	Range[1]
	kg	*kcal/kg/day*		*kg*	*kcal/kg/day*	
3601[2]	74.2±0.2	40.0	40.0	75.1±0.5	40.0	40.0
3602	73.7±0.5	40.0	40.0	73.5±0.3	40.0	40.0
3603	72.2±0.3	40.0	40.0	73.4±0.3	40.0	40.0
3604	73.2±0.9	39.7	40–39[3]	73.6±0.2	39.0	39.0
3605	72.3±0.3	40.9	40–42[4]	73.8±0.6	42.0	42.0
3606	89.4±0.5	38.9	40–38[5]	87.8±0.8	38.0	38.0

[1] Energy intake for all subjects was held constant throughout the second metabolic period. [2] Completed only 81 days of the study. [3] Energy intake for 3604 during this period was as follows: Days 1 to 35 inclusive, 40 kcal/kg/day; days 36 to 48 inclusive, 39 kcal/kg/day. [4] Energy intake for 3605 during first period was as follows: Days 1 to 21 inclusive, 40 kcal/kg/day; days 22 to 31 inclusive, 41 kcal/kg/day, and days 32 to 48 inclusive, 42 kcal/kg/day. [5] Energy intake for 3606 during first period was as follows: Days 1 to 8 inclusive, 40 kcal/kg/day; days 9 to 35 inclusive, 39 kcal/kg/day, and days 36 to 48 inclusive, 38 kcal/kg/day.

TABLE 7

The Effect of high nitrogen intake on mean blood urea and protein levels[1]

Subject group[2]	Nitrogen intake	Metabolic period	BUN	Total protein	Albumin (A)	Globulin (G)	A/G Ratio
	g		*mg/dl*	*g/dl*	*g/dl*	*g/dl*	
1		Initial	15.3±6.5	7.3±0.2	4.75±0.12	2.6±0.1	1.8±0.0
	12	1	13.7±1.2	7.1±0.4	4.55±0.15	2.6±0.3	1.7±0.2
	36	2	25.1±2.4[3]	7.5±0.4	5.21±0.18	2.3±0.2	2.2±0.2
2		Initial	15.8±2.3	7.5±0.3	4.88±0.17	2.7±0.4	1.8±0.3
	36	1	23.4±5.7[3]	7.3±0.4	4.64±0.14	2.7±0.3	1.7±0.2
	12	2	12.6±1.8	7.3±0.5	5.21±0.21	2.1±0.3	2.5±0.3

[1] Mean±SD. [2] Group 1 consists of subjects 3601, 3603, and 3605; Group 2 consists of subjects 3602, 3604, and 3606. [3] Significant difference due to diet $P < 0.05$.

the samples before refrigeration at −10°. This was an additional precaution to prevent, if any, any evolution of ammonia present during the thawing of the samples for analysis. Daily urines were weighed and tested qualitatively for protein, glucose and acetone.[4] Specific gravity, pH and osmolarity were also determined daily. After diluting the urine to an appropriate volume, aliquots were taken and frozen for later analysis. Total nitrogen (8) and creatinine (9) were determined on daily urine samples. Six-day pooled urine samples were used for the determination of 3-methylhistidine, using an amino acid analyzer,[5] 17-hydroxycorticosteroids by a modified Porter-Silber method (10, 11) and 17-ketogenics by a modified[6] method of Few (12).

Feces were collected in 3-day pools from day 4 till the end of the study and stored at −10° in a refrigerator. Fecal composites were homogenized[7] with known amounts of deionized water and analyzed for nitrogen (8).

Fasting venous blood samples from the antecubital fossa were drawn into weighed containers on days 1, 24, 49, 78, and the last day of the study, 105. A blood chemistry profile and hemogram were analyzed by a commercial laboratory.[6]

Once during each metabolic period, for 6 days and 6 nights, sweat and integumentary losses were determined as described previously (13). Scalp and facial hair, whiskers and toe- and fingernail samples were also collected and analyzed during each metabolic period by the method of Calloway and associates (13). The number of seminal discharges by the participants during each metabolic period was recorded and the amount of nitrogen lost determined using the factor 37.0 mg per ejaculate (13).

Nitrogen balance was calculated by subtracting the sum of nitrogen content in feces, urine, sweat, and integumentary losses together with the calculated amount in miscellaneous losses through such routes as blood (32.4 mg/g), saliva (0.96 mg/g) and semen (13) from the average corrected daily dietary nitrogen intake. The first 9 to 12 days of each metabolic period, assumed to be periods of adjustment to the diet and the last 6 days of metabolic period 2, when an antibiotic was administered, were not used for balance calculations.

Statistical analysis. Standard deviations were calculated for all means of a sample number greater than two. An analysis of variance was performed to test for individual differences between metabolic periods. The nitrogen balance data was also subjected to statistical tests to check whether there was a trend towards adaptation. In all statistical analyses, means were considered significantly different if $P < 0.05$.

RESULTS

With the exception of two of the participants, 3602 and 3604, who developed

[4] Combistrix, Ames Company, Elkhart, Indiana.
[5] Auto-Analyzer, Method N-11a, modified, Technicon Corporation, Channary, New York.
[6] Solano Laboratories, Berkeley, California 94705.
[7] Gifford-Wood Eppenbach Coloid Mill.

mild cases of diarrhea during the first week of the study, all the participants remained healthy throughout the study. Blood samples taken at the beginning of the study and at the middle and end of each metabolic period for routine analysis showed no abnormalities. The summaries of the mean values of some of the blood components measured are given in tables 7 and 8. Levels of all measured components, except blood urea nitrogen (BUN), remained within normal ranges under both dietary treatments during both metabolic periods. The slight changes in albumin concentration observed during the two metabolic periods were not significantly different. The differences might be due to methodological error. We believe this to be the case because the changes were related to metabolic periods and not to the diet treatment. Increasing the nitrogen intake from 12 to 36 g/day, however, caused a significant increase of BUN as expected.

Before portions of the daily collected urinary samples were taken for chemical composition analysis, urine weight, pH, specific gravity, and osmolarity were determined to assure adequate fluid intake and normal renal function. Rapid qualitative tests revealed no glucose or protein in the urine of the participants. However, it was observed that increasing or decreasing the nitrogen intake from 12 to 36 g/day or vice versa resulted in an increase or decrease in urinary weight, pH, specific gravity and osmolarity. The mean increases in urinary weight, pH and specific gravity were 328 g, 0.3 and 0.004, respectively. The mean osmolarity almost doubled, from 367 to 642 mOsm/day, as dietary nitrogen intake was increased from 12 to 36 g/day.

The concentration of total nitrogen in the urine was determined daily from day 4 until the end of the study. The effect of dietary treatment on urinary nitrogen excretion was significantly different ($P < 0.05$) between the participants. The daily variations and the effect of dietary nitrogen level on urinary nitrogen excretion by the individual participants are illustrated in figures 1 and 2. There was quite a drastic change in excretory patterns as the nitrogen intake was changed. As expected, changing the nitrogen intake from 12 to 36 g/day (group 1, fig. 1) caused a significant increase ($P < 0.05$) in urinary nitrogen output. The mean change for group 1 was from 11.08 to 32.30 g/day. It took them also about 9 to 12 days to adjust to the nitrogen load. Figure 2, graphically depicting the urinary excretory pattern for group 2 participants, shows the reverse of group 1. Lowering of nitrogen intake resulted in a proportional reduction of uri-

TABLE 8

The effect of nitrogen intake on urine composition[1]

Subject	Nitrogen intake	Metabolic period	Total weight	pH	Specific gravity	Osmolarity	Creatinine	Creatine
	g		*g*			*mOsm/day*	*mg/kg body wt.*	*g/day*
3601	12	1	2711 ±374	5.9 ±0.8	1.007 ±0.002	299 ± 46	18.96 ±0.73	1.41 ±0.06
	36	2	3060 ±270[2]	6.3 ±0.3[2]	1.009 ±0.001[2]	528 ± 46[2]	19.34 ±1.03[2]	1.45 ±0.09[2]
3602	36	1	2423 ±348[2]	6.2 ±0.2[2]	1.014 ±0.003[2]	672 ± 92[2]	21.69 ±0.80[2]	1.60 ±0.04[2]
	12	2	2205 ±337	5.9 ±0.2	1.009 ±0.003	369 ± 66	20.79 ±0.62	1.53 ±0.05
3603	12	1	2023 ±207	6.0 ±0.2	1.010 ±0.002	396 ± 37	21.43 ±0.62	1.55 ±0.04
	36	2	2355 ±338[2]	6.2 ±0.2[2]	1.015 ±0.003[2]	705 ± 86[2]	22.97 ±1.25[2]	1.68 ±0.10[2]
3604	36	1	2209 ±276[2]	6.2 ±0.2[2]	1.015 ±0.003[2]	717 ± 82[2]	21.41 ±0.62[2]	1.56 ±0.09[2]
	12	2	1959 ±298	5.9 ±0.2	1.010 ±0.003	388 ± 58	20.72 ±0.55	1.52 ±0.04
3605	12	1	2490 ±281	5.9 ±0.2	1.008 ±0.003	336 ± 36	24.98 ±0.67	1.81 ±0.05
	36	2	2988 ±392[2]	6.1 ±0.2[2]	1.010 ±0.003[2]	557 ± 76[2]	25.56 ±1.31[2]	1.89 ±0.11[2]
3606	36	1	2454 ±417[2]	6.5 ±0.2[2]	1.014 ±0.003[2]	670 ±111[2]	23.90 ±0.87[2]	21.4 ±0.08[2]
	12	2	2113 ±362	6.1 ±0.2	1.010 ±0.003	416 ± 89	23.02 ±1.12	2.02 ±0.11

[1] Mean values ±SD. [2] Significant difference due to diet $P < 0.05$.

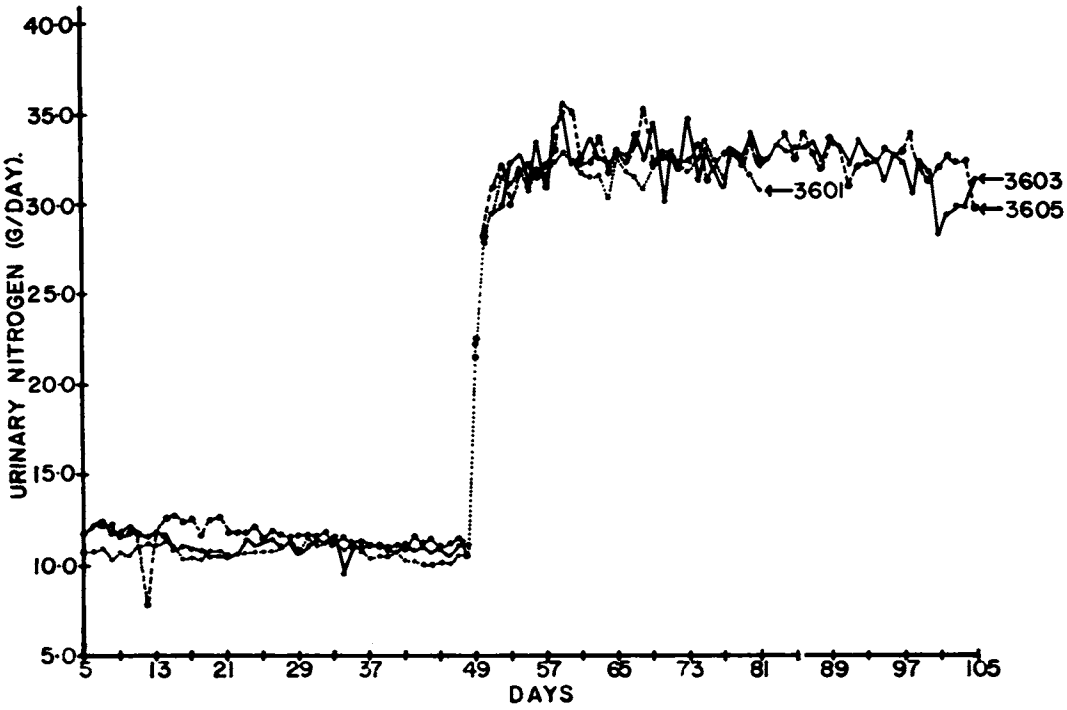

Fig. 1 Urinary nitrogen (g/day) for subjects 3601, 3603, and 3605 (group 1).

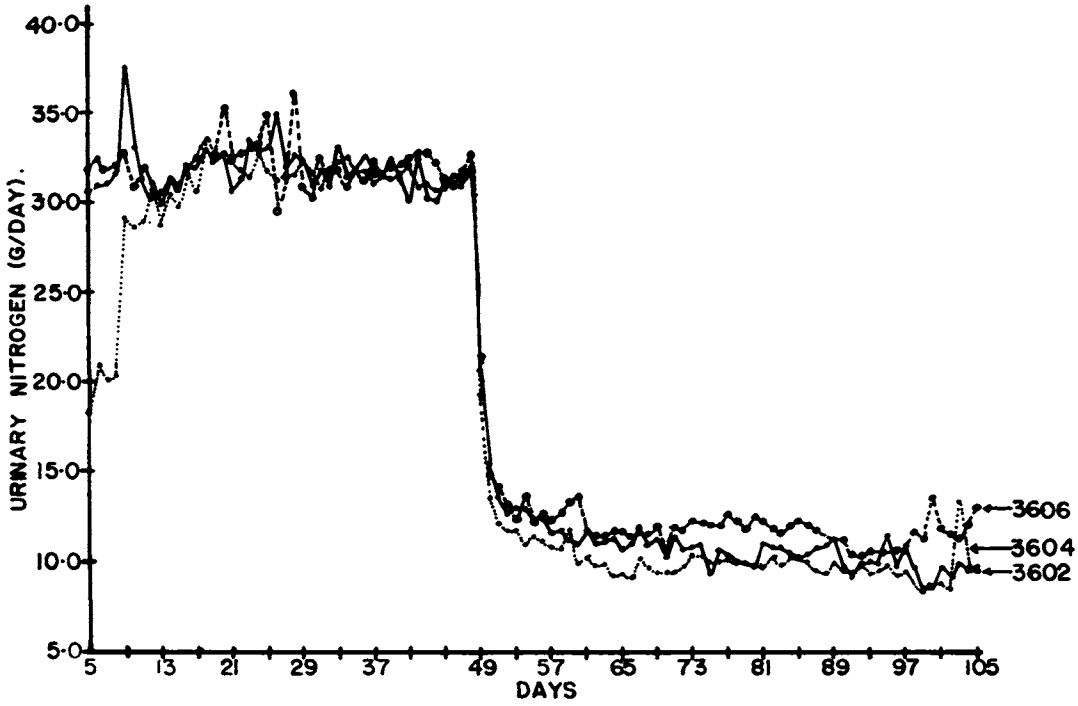

Fig. 2 Urinary nitrogen (g/day) for subjects 3602, 3604, and 3606 (group 2).

TABLE 9

The effect of nitrogen intake on mean urinary 17-hydroxycorticosteroids and 17-ketogenics excretion

Subject	Metabolic period	Nitrogen intake	17-Hydroxycorticosteroids[1]	17-Ketogenics[1]
		g/day	*mg/day*	*mg/day*
3601	1	12	28.4±1.9	16.2±1.4
	2	36	31.7±2.6[2]	17.9±1.2[2]
3602	1	36	19.6±2.1[2]	9.9±0.7[2]
	2	12	15.9±2.4	9.3±0.8
3603	1	12	17.1±1.8	9.2±0.5
	2	36	21.2±1.9[2]	11.8±1.4[2]
3604	1	36	24.9±1.9[2]	12.9±0.7[2]
	2	12	22.6±1.3	10.9±0.6
3605	1	12	20.9±1.8	11.9±1.5
	2	36	21.9±1.4[2]	13.1±0.8[2]
3606	1	36	25.1±1.8[2]	15.3±0.9[2]
	2	12	23.1±1.0	12.6±1.1

[1] Mean of seven observations ±SD. [2] Significant difference due to diet $P < 0.01$.

nary nitrogen excretion from 31.67 to 10.81 g/day and took them about the same length of time for adjustment. The effects of the mild diarrhea experienced by 3602 and 3604, especially the latter, at the beginning of the study is shown in figure 2. The greater the nitrogen lost through the fecal route, the more reduced from "normal" the urinary nitrogen excretion during this period. As the diarrhea ceased, urinary nitrogen excretion returned to the normal range characteristic of the level of nitrogen intake.

The mean daily urinary creatinine excretion increased or decreased significantly with increase or decrease in nitrogen intake by all participants even though creatine-free diets were fed. There was little or no effect on 3-methylhistidine excretion. These changes together with body weight and composition changes will be given in detail in another publication.

Urinary excretions of 17-hydroxycorticosteroids and 17-ketogenics are summarized in table 9. The participants reacted differently to the two levels of nitrogen intake. There was a significant effect of the level of nitrogen intake on the excretion of these adrenal steroids. Even though there was a significant increase in urinary excretion with increase in nitrogen intake, the lower values obtained for the 12 g

TABLE 10

The effect of high nitrogen intake on urinary and fecal nitrogen excretion and nitrogen balance in young adult males

Subjects	Metabolic period/ N level	Nitrogen intake[1]	Nitrogen output				Nitrogen balance
			Urinary[2]	Fecal	Integumentary[3]	Miscellaneous[4]	
		g/day		*g/day*			*g/day*
Group 1							
3601	1/Low	12.14	10.68±0.36	1.31±0.45	0.32	0.13	−0.29±0.61
	2/High	36.25	31.83±0.51[5]	2.48±0.37[5]	0.68[5]	0.05	1.20±0.54[5]
3603	1/Low	12.14	11.02±0.37	0.96±0.58	0.20	0.11	−0.15±0.57
	2/High	36.25	32.61±0.55[5]	1.63±0.37[5]	0.33[5]	0.07	1.61±0.69[5]
3605	1/Low	12.15	11.54±0.55	1.04±0.37	0.29	0.11	−0.76±0.75
	2/High	36.26	32.58±0.46[5]	1.72±0.58[5]	0.49[5]	0.06	1.41±0.72[5]
Group 2							
3602	1/High	36.25	31.70±0.74[5]	2.95±0.56[5]	0.52[5]	0.10	0.99±0.78[5]
	2/Low	12.14	10.48±0.47	1.59±0.24	0.25	0.06	−0.23±0.54
3604	1/High	36.25	31.30±0.89[5]	2.97±0.54[5]	0.54[5]	0.11	1.34±0.93[5]
	2/Low	12.14	9.65±0.29	2.15±0.31	0.22	0.06	0.06±0.33
3606	1/High	36.27	31.99±0.71[5]	1.89±0.28[5]	0.64[5]	0.10	1.64±0.77[5]
	2/Low	12.16	11.46±0.60	1.00±0.41	0.41	0.06	−0.78±0.76

[1] Corrected for plate wastage. [2] Mean ±SD. [3] Comprised of determined skin, sweat, hair, finger- and toenail nitrogen losses. [4] Made of calculated values from blood, saliva, and semen losses. Blood, saliva, and semen losses were weighed and the nitrogen lost calculated by using factors as in reference 13. [5] Significant difference due to diet $P < 0.05$.

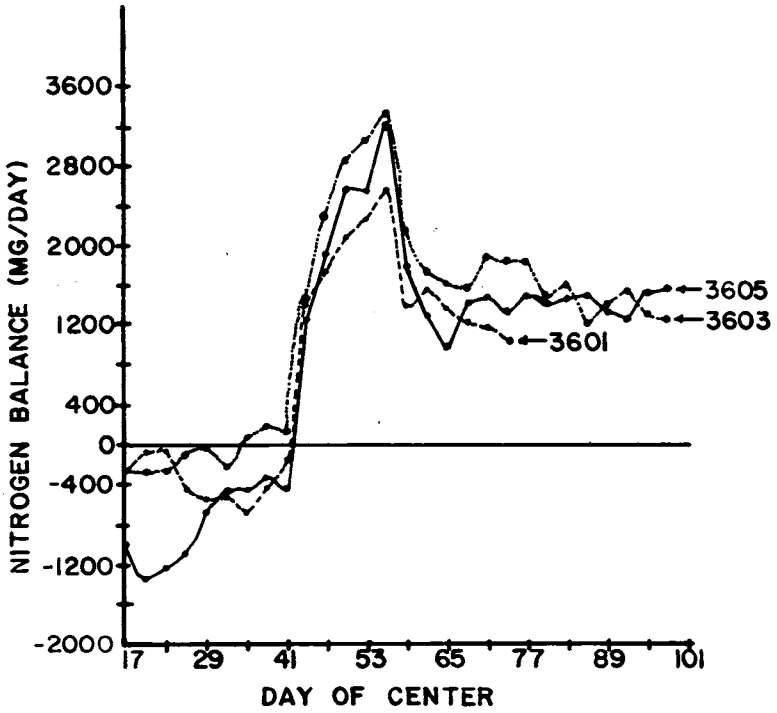

Fig. 3 Nitrogen balance (mg/day) for subjects 3601, 3603, and 3605 (group 1). "Day of Center" refers to the center or the middle point of the 5 days used for averaging.

nitrogen diet were all within normal ranges for normal healthy adult males.

Fecal nitrogen output was determined in 3-day pooled samples from day 4 until the end of the study. Fecal nitrogen output increased in all participants as nitrogen intake increased (see table 10).

Integumental and miscellaneous nitrogen losses were measured during each metabolic period. Integumental loss is the sum of nitrogen in sweat (determined in bath and laundry water), scalp and facial hair, and toe- and fingernails. Nitrogen lost through this route increased with increase in nitrogen intake in all participants (table 10). It more than doubled in three of the participants, 3601, 3602, and 3604. The order of feeding, that is, whether from 12 to 36 g nitrogen per day or vice versa, had a slight effect on integumental nitrogen lost. Group 1 participants showed a mean increase of about 84.1 (from 271 to 499 mg/day) whereas group 2 participants had a mean reduction of about 94.1 (from 567 to 292 mg/day).

Seminal discharges, collection of blood and saliva samples from the participants during the study led to nitrogen losses. These losses, grouped under miscellaneous losses (table 10), were estimated using factors previously determined in this lab (13) for each metabolic period. Nitrogen lost through this route was not affected by dietary treatment. Any observed differences between dietary treatments for each participant was due to the amount of samples collected or discharged.

Nitrogen intake, corrected for plate wastage, nitrogen output and retention for each participant by metabolic period are summarized in table 10. Significant degrees of both inter- and intra-individual variations in the groups were found but, in general, the balances appear more positive at the higher level of nitrogen intake. Figures 3 and 4, using the 5-day moving averages (14) and smoothing out random variabilities in individual data, give a clearer picture. There was a persistent positive balance at the 36 g nitrogen intake

level which was significantly different ($P < 0.05$) from the 12 g nitrogen intake. Also, there was no significant trend towards adaptation, either at the low or high nitrogen intake. The length of time it took the participants to adapt to the changeover in nitrogen intake is clearly shown. The adaptation period was about 9 to 12 days.

Table 10 gives the mean nitrogen balances for the two metabolic periods for each participant after he had adapted to the given load of dietary nitrogen. All the participants, except 3604, were in slight negative balance (-0.29 ± 0.61, -0.23 ± 0.54, -0.15 ± 0.57, 0.06 ± 0.33, -0.76 ± 0.75, and 0.78 ± 0.76 g/day, respectively) when they were fed the 12 g nitrogen diet. On the high nitrogen intake, 36 g/day, all the participants retained nitrogen ranging from 0.99 to 1.64 g/day.

DISCUSSION

Recommendation for nitrogen allowances for population groups at the national and international level are often made from data generated by studies employing the nitrogen balance technique. The accuracy of this widely used method, and consequently the adequacy of the recommended allowances, however, has been questioned repeatedly and formed the topic of many research and review papers over the last several years (15–23). One of the questions often raised about this method is the disproportionality of nitrogen retention to changes in body size, weight or composition as observed in the literature (24–26). In most studies, the higher the nitrogen intake, the higher the nitrogen retention which seems to occur even though, theoretically, in matured adults, once nitrogen requirements are met, any excess nitrogen intake should initially cause a positive balance and then finally, within a short period of time, return to balance. The discrepancy between the observed and the theoretical has been explained in a variety of ways. In addition to other reasons cited earlier in

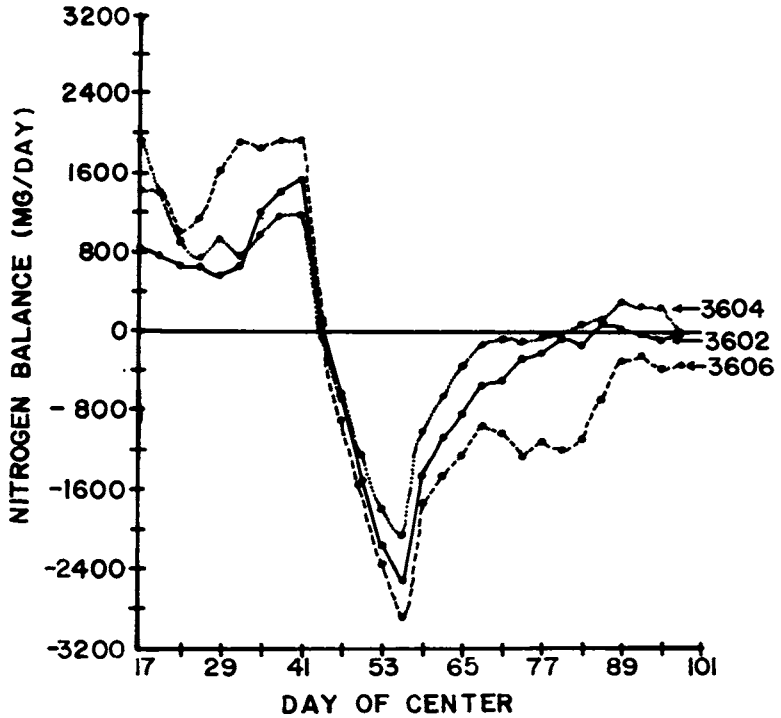

Fig. 4 Nitrogen balance (mg/day) for subjects 3602, 3604, and 3606 (group 2). "Day of Center" refers to the center or the middle point of the 5 days used for averaging.

this paper, Widdowson and McCance (27) have also suggested that the protein content of the cell may not be as fixed as claimed and that there may be a decrease in water content to accommodate an increased nitrogen retention. This paper will mainly try to verify whether, under long-term basis, increased nitrogen intake will be accompanied by an increased nitrogen retention as claimed. All necessary precautions were taken to eliminate overestimating the intake. Food spillage, plate wastage and actual nitrogen intake were determined. Frequent sampling assured minimal variation in intake. Actual nitrogen intakes by the participants ranged from 12.14 to 12.16 g/day for the 12 g nitrogen diet and from 36.25 to 36.27 g/day for the 36 g nitrogen diet. The qualities and apparent digestibilities of the proteins fed were high. Energy intake, ranging from 38 to 42 kcal/kg body weight, was sufficient to maintain body weight.

Nitrogen output was also measured as precisely as possible to avoid underestimating it. Nitrogen losses through skin, sweat, finger- and toenails, and hair growth, both facial and scalp (integumentary) and blood, saliva and semen (miscellaneous) as well as feces and urine were precisely determined.

The mean nitrogen balance was slightly negative for most participants when fed the 12 g nitrogen diet if balance is taken as a line. However, individual variability, as has been shown in many human studies, was so large that practically all the participants can be considered in balance if balance is considered as an area, 0.0 ± 0.5 g. This brings up a very critical point which is often overlooked in interpreting metabolic balance data. Even though this study was conducted for a relatively long period with an adequate nitrogen intake (more than recommendation) and sufficient energy intake for efficient utilization of the amount of nitrogen fed (body weight was maintained or increased), the intravariability of each participant was far more than the balance. From the practical point of view it would be more appropriate, therefore, to consider balance as an area, as already mentioned, rather than a line. Even though statistical tests (*t*-test and Wilcoxon) showed that all these negative values are not significantly different from zero, the number of participants makes it impossible to categorically conclude that they were in balance (by a line method).

Changing the nitrogen intake from low to high as done in group 1 caused a short period (6 to 9 days) of huge positive balance. This has been observed in both animals (28) and men (29–31). Changing protein intake of rats from low to high causes an increased concentration of total plasma amino acids on the first few days due to the low activities of amino acid-degrading enzymes and the efficiency of readsorption of amino acids by the kidneys. As the activity of the catabolic enzymes rises, the concentrations of the plasma amino acids fall and urea synthesis and excretion increase (28). In this study, as the urinary nitrogen increased, nitrogen retention decreased from a mean of 7.9 g to 1.6 g/day which persisted for as long as the high-nitrogen diet was fed.

The participants in group 2 whose dietary treatment changed from high to low showed the reverse of the reactions observed in group 1 participants. The changeover resulted in a large nitrogen loss for a short time followed by a drastically reduced urinary nitrogen excretion. Mean nitrogen balance increased from -6.0 to 0.65 g/day.

Energy intakes by the participants were increased or decreased accordingly to maintain body weights during the first metabolic period. However, during the second metabolic period, energy intakes remained constant despite changes in body weight. It was observed that under both dietary regimens, after an initial change, body weights tended to stabilize. Increasing or decreasing the nitrogen intake from 12 to 36 g or from 36 to 12 g/day, with an energy intake remaining constant, resulted in an increase (in group 1) or decrease (in group 2) in mean body weight for all participants. This aspect of the results will be treated fully in another paper.

The extra nitrogen lost through the urine during the adaptation period has been given many names including "labile protein." Munro (32) defined it as a protein which is rapidly dissipated on a low pro-

tein intake and rapidly restored on a high one. This has been estimated in the human adult by many investigators. From experiments on infant and adult human subjects (31, 33–35), dogs (36) and rats (37), it has been concluded that labile protein ranges from less than 1% to about 5% of the total body protein. Calculating the labile nitrogen lost or gained in this study as the difference between the urinary nitrogen excreted during the period of adaptation and the nitrogen excreted after a steady state had been reached at the new level (33), it was observed that the participants in group 1, using the factor 34 g N/kg fat-free body mass (38), gained an average of 16.32 g N (16.33, 16.09, 16.54 g, respectively), constituting about $0.85 \pm 0.09\%$ of the fat-free body mass nitrogen or 0.22 ± 0.1 g/kg body weight, whereas group 2 participants lost an average of 29.99 g N (34.42, 28.54, 27.02 g, respectively) or $1.41 \pm 0.31\%$ of the fat-free body mass nitrogen or 0.38 ± 0.8 g/kg body weight. It seems that the level of labile nitrogen gained or lost was strongly influenced by the level of the previous nitrogen intake. On body weight basis, the difference between the two groups was significant ($P < 0.05$). Whether this difference was due to real changes in labile pool size is questionable; it could merely be due to variability between the small number of participants.

All the participants exhibited strong positive nitrogen balances when they were fed the 36 g nitrogen diet. This positivity persisted for as long as they were fed this diet. The data did not show any downward trend with time. The longest time the participants were fed 36 g nitrogen per day was 57 days. Forbes (6) suggested that physiological adjustments to dietary changes might be slower and consequently longer than previously assumed. It is not possible to state from this data if or when the observed positive nitrogen balance will return or drift to a normal level.

Underestimation of total nitrogen output is one of the often cited reasons for the observed increase in nitrogen retention in mature adults fed high-nitrogen diets. In most studies in the literature, especially those showing very large retentions on intakes less than 36 g as fed in the present study, this reason might partially explain the observance. Underestimation of output was avoided in this study. One source of nitrogen loss not measured in this study was the amount of fecal nitrogen lost on toilet paper. Previous studies by Calloway, Odell and Margen (13) did show, however, that the amount of nitrogen lost through this route is inconsequential—from 3 to 8 mg/day on nitrogen intakes ranging from 12 to 96 g/day. Ammonia nitrogen lost through the breath was not directly determined in this study. The magnitude of this loss, however, was determined spectrophotometrically [8] by the indophenol color reaction as modified by Chaney and Marbach (39) in a random sample of laboratory personnel (9 males, 25 to 53 years old and weighing between 61.4 and 81.8 kg) whose daily nitrogen intake was about 12 g. The nitrogen lost through this route was even lower (3.73 ± 0.77 mg/day) than previously measured (13). The difference is due, most likely, to the different methods. Since there is no increase in ammonia nitrogen excretion with increased nitrogen intake,[9] the amount of nitrogen lost through this route is far less than necessary to explain the magnitude of the observed nitrogen retained by the participants in this study. Loss of ammonia through the skin was not measured but would not have been expected to account for anything approaching the magnitude of the positive nitrogen balance observed.

The difficulties and discrepancies associated with the nitrogen balance method have caused many investigators to question its validity as a sole criterion for estimating the amount of nitrogen which is necessary to maintain an individual in optimal health. Yoshimura (15) has therefore suggested the use of other physiological parameters, such as measurement of adrenal function. The effect of the levels of nitrogen fed in this study on adrenal function was therefore measured. With the high nitrogen intake, there were slightly higher levels of urinary 17-hydroxycorticosteroids and 17-ketogenics. It is doubtful, however, that this has any relationship to nitrogen re-

[8] Zeiss Spectrophotometer PMQ II.
[9] Calloway, D. H. Private communication.

quirements. It might merely be an expression of the body's homeostatic mechanism to maintain itself at the proper state of equilibrium in the presence of an enormous amount of dietary nitrogen intake. At this very high level of nitrogen intake, which might constitute a stressful situation for the body, it is not unreasonable to suspect an increased secretion of cortisol to enhance deamination and increased gluconeogenesis. One side effect observed in this experiment, as in many other works, is the induction of hypercalciuria by the high nitrogen intake. This could not be explained by changes in either urinary hydroxyproline or parathyroid hormone levels. Full details of this aspect of the work will be published elsewhere.

The data obtained in this study, considering all the facts, point to the conclusion that nitrogen intake of 36 g/day results in a persistent nitrogen accretion of about 1.6 g/day that does not appear to be due to methodological error.

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