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PROGRESS REPORT

Work in three areas of research is covered in this report.

1. Dietary Protein and Uric Acid Metabolism in Normal Man*

   A. <u>Uric acid pool size and turnover</u>

   Increasing dietary protein intake has been reported to raise urinary uric acid excretion in humans. In our laboratory, a direct relationship between dietary protein intake and uric acid excretion was shown with strictly purine-free, formula diets containing up to 96 g nitrogen per day. The increased uric acid produced by the very high protein diets could have reflected similarly increased <u>de novo</u> purine synthesis. However, enhanced renal elimination might have been a contributing factor since blood uric acid levels were not elevated. To determine the causes of the increased uric acid excretion produced by raising the protein intake, uric acid miscible pool and turnover rate were measured with ^{15}N labeled uric acid in men fed diets containing no protein, about 390 g of protein, and 80 g of protein with or without added yeast ribonucleic acid (RNA). The high protein and RNA levels were selected to produce the same urinary uric acid excretion on the basis of results from previous experiments.

   The proteins from egg white in the control diet and from a mixture of egg white, soy protein, and casein (1:2:2) in the high protein diet were incorporated into liquid formulas containing carbohydrate, fat, and macrominerals. Since the mineral composition of the different protein sources varied, salts were added to the protein-free and control formulas to match the mineral content of the high protein formula which was adjusted to maintain urine pH nearly alkaline and promote solution of uric acid. The sulfur content of the diets was not equalized because of the laxative properties of most sulfate salts. Vitamins and trace minerals in the quantities reported previously were given in capsule form. Each basic formula contained 2400 calories but the men with higher requirements received a liquid caloric supplement to maintain their weights. Six healthy male volunteers participated in the experiment during 9 weeks of confinement to our metabolic unit.

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*Abridged from "Dietary protein level and uric acid metabolism in normal man." J. Nutr. 100: 249-261, 1970.

Analyses were performed on daily urine samples throughout the entire experiment for total nitrogen by the micro-Kjeldahl method, uric acid by an enzymatic-spectrophotometric method, and creatinine by the automated alkaline picrate method. During the latter half of each period, analyses were made of 3-day pools for urea nitrogen by the automated carbamido-diacetyl reaction. Total alpha amino nitrogen and ammonia were determined on selected days with HCl used as preservative. A rough quantitative estimate of individual amino acids was made by thin-layer chromatography on cellulose plates using diethylamine, n-butanol, acetone, and water (6:30:30:15) for the first dimension (two times) and formic acid, sec-butanol, and water (1:6:2) for the second dimension.

Fasting samples of blood serum taken on the last day of each metabolic period were assayed by standard automated procedures for creatinine, urea nitrogen, uric acid, and glucose. During the second half of each period, several additional fasting samples were analyzed for uric acid and creatinine.

Once each metabolic period, some of the men received intravenously uric acid $1,3-^{15}N$ (95 to 99% enriched). Uric acid infusions lasting 10 to 20 min were started in the morning with the men fasting in periods 1 and 4 and 2 hrs after breakfast in periods 2 and 3. The I.V. was flushed with 5% glucose solution and a second blood sample taken from the other arm exactly 10 min after completing the infusion. Urine was collected in 12-hr periods for 2 days after injecting uric acid in periods 1 through 3 and for 3 days in period 4. Miscible pool size and turnover rate were calculated from the equation $A = a(\frac{Ii}{Io}-1)$ where A = miscible pool, a = dose of uric acid injected, Io = concentration of isotope at zero time, and Ii = concentration of isotope injected.

Urine volume (weight) averaged 2250 to 2450 g per day on the protein-free and control diets and increased to 3640 g per day with the high protein diet. In period 4 (RNA feeding), when fluid intake was increased intentionally to avoid precipitation of uric acid, mean urine weight was 2650 g per day. Mean specific gravity was in the range of 1.008 to 1.014 throughout the experiment. Urinary total solute excretion averaged 604, 842, and 864 mOsm per day on the protein-free, control, and RNA diets, respectively, and increased to 2455 mOsm with the high protein diet. Urine pH was in the 7.0 to 7.5 range on the protein-free, control, and RNA diets and fell about one unit with the high protein diet.

Table 1 gives the urinary excretion of all nitrogenous compounds determined. Daily output of the three compounds usually varied together, indicating fluctuation in 24-hr urine production or sample collection and preparation rather than analytical error. Except for creatinine, all urinary nitrogen compounds varied directly with the protein intake, with the major changes occurring in urea. Excretion of total alpha amino nitrogen was low on the protein-free and control diets but rose 2.5 times when the protein intake was increased 5 times. The elevated alpha amino nitrogen was mainly due to increased excretion of alanine, glycine, serine, glutamine, alpha amino isobutyric acid, histidine, and methyl histidine while taurine and cystine excretion decreased. Urinary ammonia nitrogen was closely correlated with protein intake and always accounted for 2% of urinary nitrogen output.

Urinary uric acid decreased slightly and blood uric acid rose slightly on the protein-free diet, while the high protein diet elevated urinary uric acid 2.5 times above the control period without influencing serum levels. The elevation in urinary uric acid produced by a 5-fold increase in protein intake was the same as that produced by adding 4 g yeast RNA per day to the control diet. However, RNA feeding increased the serum uric acid level in four subjects by 2.5 to 4.5 mg per 100 ml and in one man by 6.8 mg per 100 ml.

Clearances of creatinine and uric acid were calculated from 24-hr urine and fasting blood values. Creatinine clearance (C_{cr}) averaged 90, 96, 110, and 96 ml per min on the protein-free, control, high protein, and RNA diets, respectively. Although clearance changes were not large, they were observed consistently in all subjects. Uric acid clearance (C_{ur}) increased from a control mean value of 5 ml per min to 8 ml per min with RNA feeding and to 14.5 ml per min on the high protein diet. Using endogenous creatinine clearance as an estimate of glomerular filtration rate, the uric acid filtration rate (F_{ur}) defined as C_{cr} x P_{ur} was similar (5.0 and 5.8 mg per min) on the control and high protein diets. Since clearance changes may be due to changes in either reabsorption or secretion of uric acid, we prefer the term "net renal retention," defined as ($F_{ur} - U_{ur}V$), to "apparent reabsorption." Net renal retention of uric acid was only 86% of the filtered load on the high protein diet, compared to a range of 94 to 96% of the filtered load on the protein-free and control diets. The net urate retention on the RNA diet averaged 91% of an 8.2 mg per min filtered load.

Table 4

Urinary Nitrogenous Components With RNA and
Different Levels of Dietary Protein

Subject	Diet[1] g N/day	Period	Total Nitrogen[2] g/day	Creatinine[2] g/day	Uric acid[3] mg/day	Urea Nitrogen[4] g/day	Total Alpha Amino Nitrogen[5] g/day	Ammonia Nitrogen[5] g/day
1	0.9	1	2.34 ± 0.31[7]	1.86 ± 0.14[7]	373 ± 62[7]	0.94	0.12	0.04
23 yr[6]	13	2	8.70 ± 0.34	1.79 ± 0.05	462 ± 26	6.16	0.19	0.22
81.0 Kg	62	3	51.80 ± 3.97	1.85 ± 0.08	1168 ± 36	48.02	0.48	1.17
182 cm	13 + RNA	4	11.63 ± 0.74	1.85 ± 0.05	1125 ± 37	8.49	---	---
2	0.9	2	2.23 ± 0.28	1.32 ± 0.04	265 ± 16	1.19	0.09	0.06
24 yr	13	3	9.81 ± 0.46	1.29 ± 0.04	327 ± 14	7.67	0.12	0.19
59.5 Kg	62	1	52.91 ± 3.60	1.50 ± 0.09	1019 ± 79	48.64	0.38	1.24
169 cm	13 + RNA	4	10.96 ± 0.53	1.29 ± 0.03	912 ± 74	8.79	---	---
3	0.9	3	2.10 ± 0.40	1.18 ± 0.06	249 ± 26	1.14	0.10	0.02
24 yr	13	1	8.94 ± 0.64	1.25 ± 0.08	300 ± 31	6.57	0.12	0.14
56.0 Kg	62	2	51.74 ± 2.54	1.24 ± 0.06	1035 ± 35	47.69	0.34	0.92
172 cm	13 + RNA	4	8.99 ± 0.49	1.14 ± 0.02	896 ± 34	6.92	---	---
4	0.9	3	1.88 ± 0.08	1.43 ± 0.04	354 ± 12	0.79	0.09	0.04
31 yr	13	2	10.17 ± 0.54	1.51 ± 0.07	416 ± 26	7.73	0.12	0.18
66.5 Kg	62	1	50.59 ± 2.15	1.59 ± 0.09	1097 ± 92	46.76	0.26	1.01
180 cm	13 + RNA	4	10.58 ± 1.00	1.43 ± 0.12	1186 ± 134	7.50	---	---
5	0.9	2	2.28 ± 0.16	1.86 ± 0.10	334 ± 19	0.94	0.10	0.04
26 yr	13	1	10.25 ± 0.51	1.95 ± 0.12	435 ± 26	7.20	0.14	0.20
84.5 Kg	62	3	52.18 ± 1.51	1.94 ± 0.07	1152 ± 106	46.57	0.35	1.24
182 cm	13	4	12.92 ± 0.58	1.95 ± 0.10	---	10.08	---	---
6	0.9	1	1.97 ± 0.17	1.37 ± 0.05	262 ± 15	0.87	0.07	0.04
24 yr	13	3	9.88 ± 0.64	1.34 ± 0.04	390 ± 27	7.78	0.10	0.19
56.0 Kg	62	2	53.06 ± 0.79	1.36 ± 0.05	1129 ± 71	48.60	0.23	1.09
169 cm	13 + RNA	4	10.99 ± 0.08	1.34 ± 0.01	1097 ± 64	8.65	---	---
Mean	0.9		2.13	1.49	306	0.98	0.09	0.04
	13		9.62	1.52	388	6.98	0.13	0.18
	62		52.05	1.58	1095	47.71	0.34	1.11
	13 + RNA		10.63	1.50	1043	8.40	---	---

[1]Diets: 0.9 (protein-free); 13 (control); 62 (high protein); 13 + RNA (control + RNA). [2]Analyzed daily; n = 9, periods 1 and 2; n = 12, period 3. [3]Analyzed daily except when preservative (HCl) added: n = 6, periods 1 and 2; n = 8, period 3. [4]Analyzed on 3-day pools: n = 3, periods 1 and 2; n = 4, period 3. [5]Selected one-day analyses: n = 2, period 1; n = 3, periods 2 and 3. [6]Age, weight, and height of subjects.

Data on uric acid turnover and miscible pool for all men are given in Table 2. The results show considerable individual variation but the influence of each dietary treatment is defined extremely well. Miscible uric acid pools of 767, 1060, and 1485 mg found with the control diet agree with the mean of 1220 mg obtained from published values on normal subjects. The high protein diet produced no change in urate pool size while ingestion of 4 g RNA per day doubled it. Uric acid turnover rate (the slope of the line relating $\log_e$ isotope excretion and time) was doubled by feeding a high protein diet and increased only very slightly by feeding RNA compared to the control diet. Consequently, turnover time was reduced from a range of 1.5 to 1.9 days on both 13 g N diets to a range of 0.74 to 0.99 days on the high protein diet.

The percentages of the daily uric acid turnover excreted in urine were 46 to 78% on the control diet, 68 to 75% on the RNA diet, and 73 to 90% on the high protein diet. The actual amount of uric acid which escaped renal elimination, i.e. the difference between daily turnover and urinary excretion, was similar (100 to 300 mg per day) on the control and high protein diets and probably represents bacterial degradation. This value increased to a range of 357 to 527 mg per day with RNA feeding, in proportion to individual differences in expansion of urate pool size.

Thus, according to our isotope studies, the identical uric acid excretion (1000 to 1200 mg per day) produced by feeding either 62 g N or 4 g RNA arose by different mechanisms. We observed consistently that addition of RNA to the control diet expanded the miscible urate pool and elevated serum uric acid but caused relatively small increases (15 to 25%) in uric acid turnover rate. On the other hand, the increased urinary uric acid observed with the high protein diet was the result of a 2-fold increase in turnover rate and no change in pool size.

Renal excretion of uric acid is the result of reabsorption of a large proportion (possibly 100%) of the filtered load and tubular secretion of a small amount of that reabsorbed. In the present study, a drastic elevation in protein intake and amino acid catabolism was accompanied by increases in urine volume, solute load, and creatinine clearance. Using creatinine clearance as a measure of GFR and calculating the filtered load and net renal retention of uric acid, it appears that some consequence of the high protein diet caused renal elimination of a higher proportion of the filtered uric acid. The high protein diet increased excretion of free amino acids

Uric Acid Miscible Pool and Turnover on Diets Containing Different Levels of Protein and RNA

Table 2   6

Diet	Subject	Body Weight	Serum Uric Acid	Dose Injected	I_i = Isotope Injected	I_o = Antiln of Intercept	A = Miscible Pool [1]	K = Slope	KA = Turnover	U = Urinary Uric Acid	Difference KA – U	$\dfrac{U}{KA}$	Turnover Time
		Kg	mg/100ml	mg	Atom % excess		mg	day^{-1}	mg/24 hrs	mg/24 hrs	mg/24 hrs	%	days
Control	3	56.0	5.2	74.0	47.5	3.10	1060	-0.61	647	300	347	46	1.92
	5	84.5	5.4	73.5	47.5	2.24	1485	-0.52	772	435	337	56	1.64
	6	56.5	4.8	63.0	49	3.72	767	-0.65	498	390	108	78	1.54
High Protein	1	81.0	5.3	64.8	49	2.42	1247	-1.04	1297	1168	129	90	0.96
	2	59.5	4.9	74.5	47.5	2.91	1141	-1.22	1392	1019	373	73	0.82
	3	56.0	5.7	62.7	49.5	2.83	1034	-1.14	1179	1035	144	88	0.88
	5	84.5	6.2	65.8	49	2.10	1469	-1.01	1484	1152	332	78	0.99
	6	56.5	4.5	57.6	49.5	2.76	975	-1.35	1316	1129	187	86	0.74
Control + RNA	1	81.0	8.0	100	49	2.29	2040	-0.81	1652	1125	527	68	1.23
	2	59.5	9.4	107	49	2.72	1820	-0.78	1420	912	508	64	1.28
	3	56.0	12.0	111	49	2.43	2127	-0.66	1404	896	508	64	1.51
	6	56.0	8.1	105	49	3.05	1580	-0.92	1454	1097	357	75	1.09

[1] $A = a \left[\dfrac{I_i}{I_o} - 1 \right]$

which are actively reabsorbed, and it is possible that some amino acids competed more favorably than uric acid for reabsorption. Tubular secretion is known to be reduced in the presence of structurally dissimilar compounds such as lactate, betahydroxy butyrate, and pyrazinamide, but no information is available on uric acid secretion.

Dietary proteins and their metabolites could also affect uric acid production by altering the concentrations of substrates for purine synthesis and by influencing feedback controls on metabolism of purine-containing compounds. Evidence for regulation of purine biosynthesis by feedback inhibitory mechanisms has been obtained directly from bacteria and from mammalian tissue studied in vitro and indirectly from human studies. Adenosine monophosphate (AMP) and guanosine monophosphate (GMP), arising either de novo or from degradation of nucleic acids, trinucleotides and dinucleotides, control their own synthetic rate at the phosphoribosylpyrophosphate (PRPP) amidotransferase step, initiating purine ring formation, as well as by inhibiting conversion of inosine monophosphate (IMP) to adenine and guanine mononucleotides.

A rapid rate of purine synthesis requires a large intracellular glycine supply, but it seems unlikely that an increased dietary glycine intake would stimulate purine production. On the high protein diet, the glycine intake was 10 g per day, only slightly more than the dosage (100 mg per kg body weight) commonly fed in isotope incorporation studies without demonstrable increases in urinary uric acid excretion. Since glycine enters the purine synthetic pathway beyond the rate-limiting step, a deficiency but not an excess of this amino acid might influence the reaction. Glutamine, however, does participate in the rate-limiting reaction of purine synthesis, but regulation seems to depend on inhibition by end-products rather than by substrates present prior to completing the purine ring. In the absence of extensive information on control of purine metabolism in humans, the importance of glutamine should not be discounted.

Several human studies have indicated that purine metabolism is responsive to exogenous as well as endogenous sources of key compounds. In the present study, dietary RNA should have provided mononucleotides for suppressing de novo purine synthesis. Evaluation of the extent of suppression based on uric acid excretion is difficult because an unknown quantity of uric acid is degraded by intestinal bacteria. However, on the basis of the turnover data, it did not appear as if the production of uric acid from

endogenous precursors was reduced. Our results showed that 600 to 700 mg uric acid per day is produced with a 75 to 80 g protein, purine-free diet. Assuming that each gram of RNA can be degraded to a maximum of 175 mg uric acid, 4 g RNA should have given rise to 700 mg uric acid. If there were no suppression of de novo purine synthesis by exogenous RNA, total daily urate production would have been 1300 to 1400 mg. The fact that the men on the RNA supplement actually produced 1400 to 1600 mg uric acid (Table 2), with about 1000 mg appearing in the urine, does not support a concept of inhibition of purine synthesis by exogenous nucleic acid.

It seems reasonable that ingesting an unusually large quantity of protein could stress a number of homeostatic mechanisms including regulation of purine metabolism. In rats, the activities of many amino acid catabolic and gluconeogenic enzymes increase with increased protein intake. Regardless of whether ATP is a co-factor for a reaction such as urea formation, both ATP and GTP are required for protein synthesis. The synthesis of enzymes of protein metabolism as well as possible increases in turnover rates of non-enzymatic proteins would increase cellular energy requirements. Many reactions produce AMP-enzyme complexes, making less AMP available for feedback control and retarding ATP synthesis. Reducing either ATP concentration or AMP supply theoretically should diminish the potential for control over nucleotide production and subsequent degradation.

Feeding a high protein diet presented a number of alternatives which may have acted independently or in concert to produce adequate substrate for increased uric acid production and to relax controls on purine degradation. Regardless of the underlying mechanisms, we have shown that the regulation of purine metabolism is interrelated very strongly with the nutritional environment of the organism.

B. Urea- and glucose-induced diuresis

A short experiment was performed with four men to determine the effect of water diuresis associated with a high protein diet on uric acid excretion. Men receiving a low protein diet (6 g N per day) were given,

intravenously, 4 liters of solutions containing either 5% glucose or 5% glucose plus 160 g urea over 12 hr periods on 4 days. The quantity of urea and magnitude of diuresis were selected to match results obtained in a previous study when the men received 96 g protein per day.

Both the glucose and glucose plus urea infusions increased daily urine volume from about 2400 ml to 5 to 6 liters. Uric acid excretion was not affected by glucose excretion, i.e. by diuresis per se and was affected only slightly when a urea load was also imposed. With administration of 75 g urea nitrogen, uric acid excretion was increased approximately 100 mg per day over the preinfusion mean value of 350 mg.

This study indicates that elevated blood and urinary urea concentrations may enhance renal uric acid output. The increase in urinary uric acid was not large (less than 30%) although urine volume was greater than in the long-term study (Part A). However, with uric acid formation stimulated by a high protein diet, in contrast to urea loading, the total urate burden to the kidney was increased by about 700 mg per day and a urea effect may have been quantitatively more important. How urea, which is eliminated by passive diffusion, could affect either reabsorption or secretion of uric acid is not clear.

C. **Suppression of uric acid formation from dietary nucleic acid with allopurinol***

The high levels of serum and urinary uric acid found in gout and some other diseases can be reduced by allopurinol, a hypoxanthine analog, which inhibits xanthine oxidase. The block in uric acid formation causes increased excretion of hypoxanthine and xanthine; but, since allopurinol

*Abridged from the paper of the same title, Am. J. Clin. Nutr. 22: 1426-1428, 1969.

also has a feedback inhibitory action on the initial step of purine synthesis, the reduction in uric acid output exceeds the increased excretion of its precursors. Thus, allopurinol is used effectively to control uric acid levels in blood and urine in diseases where metabolic defects enhance endogenous purine synthesis.

Urinary and serum uric acid levels are elevated also by exogenous purines. These occur in small amounts in normal diets but are abundant in the "single-cell protein sources" (algae, bacteria, yeasts) being considered for bioregenerative space systems and might constitute a bar to their use as food for the crew. We were led therefore to inquire if allopurinol would limit the formation of uric acid from dietary precursor ribonucleic acid.

Six healthy men received in four equal meals a formula containing 8 g yeast ribonucleic acid (RNA) and 50 g casein per day and nutritional supplements as reported previously. Four subjects received 100 mg allopurinol daily on the days indicated in Table 3 and Figure 1. Two men were controls and received RNA but no drug.

Daily 24-hr urine collections and fasting blood serum taken every third day were assayed for uric acid by the phosphotungstate reduction method using a Technicon Autoanalyzer. Urinary hypoxanthine + xanthine was assayed according to a revision of the enzymatic-spectrophotometric method for uric acid.

In previous experiments in this laboratory, a 50-g casein diet without yeast RNA produced a urinary uric acid excretion of about 400 mg per day with serum uric acid in the 4 to 6 mg per 100 ml range. As shown in Table 3 and Figure 1, addition of 8 g yeast RNA daily to the purine-free diet gave 3- to 5-fold increases in urinary uric acid and 2- to 3-fold increases in serum uric acid over values obtained with a 50-g protein, purine-free diet. When 100 mg per day of allopurinol was given to men receiving 8 g yeast RNA, serum uric acid approached the high normal range, 6 to 8 mg per 100 ml, and urinary excretion was reduced to 700 to 900 mg uric acid per 24 hrs (Table 3). During allopurinol treatment, urinary hypoxanthine + xanthine excretion was increased from 10 mg per 24 hrs to about 500 mg per 24 hrs. Figure 1, in which daily urinary data are given for two subjects, shows an immediate rise in hypo-xanthine + xanthine excretion and a slightly slower decline in uric acid excretion during continued administration of allopurinol. Fasting blood serum taken 2 days after initiating allopurinol treatment also indicated the suppression of uric acid formation. After discontinuing allopurinol, approx-imately 2 days were required for the drug effect to wear off completely,

Table 3   11

Effect of Yeast RNA and Allopurinol[a] on Serum Uric Acid and Urinary Oxypurines in Humans

| Subject	Age	Weight	Day of Study[b]	RNA[c]	Allo-purinol	Serum Uric Acid	Urinary Oxypurines		
							Uric Acid	Hypoxanthine + Xanthine[d]	Total Uric Acid Equivalents[e]
		kg				mg/100 ml	mg/24 hrs		
2	22	79	pre-test	—	—	4.3	414	—	—
			1-3	+	—	12.0	1703	18	1721
			4-6	+	+	6.8	905	503	1408
3	23	68	pre-test	—	—	5.8	365	—	—
			1-3	+	—	13.8	1702	11	1713
			4-6	+	+	7.7	810	376	1188
1	28	90	—	—	—	—	—	—	—
			1-3	+	—	10.8	1244	10	1254
			4-6	+	—	9.0	1606	12	1618
4	30	69	pre-test	—	—	5.6	277	—	—
			1-3	+	—	11.8	1541	9	1550
			4-6	+	—	12.8	1399	10	1409

[a] 'Zyloprim' brand allopurinol, 100 mg/day.

[b] Urine samples from days 1-3 and 4-6 were analyzed daily and reported as 3-day averages.

[c] Yeast ribonucleic acid, 8 g/day.

[d] Expressed as uric acid equivalents. Equivalents equal mg uric acid formed from total hypoxanthine and xanthine.

[e] Sum of uric acid and uric acid equivalents derived from hypoxanthine and xanthine.

Figure 1   12
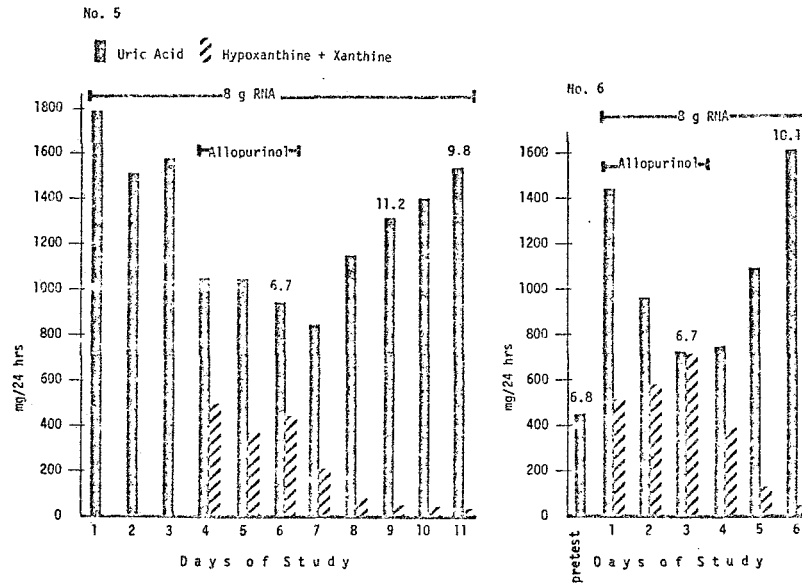
Daily urinary oxypurine excretion for two subjects.
Numbers above bars refer to serum uric acid in mg/100 ml.

according to the urinary oxypurine data. Fasting serum uric acid levels also returned to pre-treatment levels in 2 days.

Total oxypurine excretion (the sum of actual uric acid and hypoxanthine + xanthine expressed as uric acid equivalents) by subjects 2 and 3 during the period of allopurinol treatment was 300 to 500 mg less than when the same subjects were untreated. This suggests that even in the presence of a large exogenous purine load allopurinol suppresses uric acid formation from purines synthesized endogenously. However, the possibility of increased excretion of undetermined purine degradation products cannot be excluded.

2. Variation in Fasting and Postprandial Amino Acids of Men Fed Adequate or Protein-Free Diets*

This report presents fasting and postprandial serum amino acid patterns of healthy men fed precise formula diets containing either an adequate amount or no protein. Data presented here were compiled from six studies conducted in our metabolic unit between 1965 and 1967. The subjects were voluntary, all males, in good health, between 20 and 39 years of age. Body weights ranged from 59.2 to 105.6 kg and height from 168.0 to 199.0 cm. The men had similar exercise schedules, were ambulatory and confined to a metabolic unit under supervised nursing care.

Twenty-six subjects received a diet containing 12 g of egg white nitrogen (control diet). Length of time the diet was given before blood was sampled ranged from one to 84 days. Sixteen subjects were given a protein-free diet (containing between 0.51 and 0.83 g of nitrogen per day). They had been fed the diet for periods ranging from one to 18 days when blood samples were obtained. In cases where more than one serum sample was available from a given subject-treatment, the average of the values found in all of his samples was used for subsequent comparisons between individuals and diets.

The diets were consumed in four equal feedings at about 8:30 a.m., 12:30, 5:30, and 9:30 p.m. Fasting blood samples were drawn at 7:30 a.m., 10 hrs after the last meal. Postprandial samples were taken from six men fed the protein-free diet and from eight during the control treatment. Collection times were 1-3 hrs after the subject consumed either breakfast or lunch.

Amino acids were measured in picric acid extracts of serum by ion-exchange chromatography on a Spinco-Beckman analyzer. Tryptophan is not reported because values were frequently below the level of reliable quantification. Results were evaluated statistically using the paired t-test. Data are tabulated for entire groups but inference is based on comparisons of paired data. Paired comparisons available for the various subgroups were: control vs. protein-free, fasting n = 13, fed n = 6; control, fasting vs fed, n = 8; protein-free, fasting vs fed, n = 6.

In Table 4 are recorded fasting concentrations of the serum amino acids in 13 different subjects whose control (12 g nitrogen) patterns were measured

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*Abridged from the paper of the same title, Am. J. Clin. Nutr. 22: 1577-1583, 1969.

Table 4 15

Variation in fasting serum free amino acid concentrations of men maintained on a control diet containing 12 g egg white nitrogen or a protein-free diet

Subject No.	0202	0201	0203	0101	0103	0503	0506	0206	0903	0805	0806	0801	0301	Control 26[a]	No protein 16[a]
	Observations within individuals maintained on adequate diet													Groups	
Number of samples	7	5	5	4	3	2	2	3	3	3	3	3	2		
Amino acids	Mean	Mean	Mean	Mean	Range	Range	Range	Range	Range	Range	Range	Range	Range	Mean	Mean
Threonine	190 ± 52	261 ± 24	159 ± 78	265 ± 33	212–276	103–120	97–140	138–174	156–216	68–94	169–179	144–166	161–205	185 ± 64	113 ± 44
Valine	341 ± 48	311 ± 16	278 ± 81	326 ± 39	247–298	244–271	139–294	247–380	260–328	197–242	218–280	268–363	281–343	295 ± 54	195 ± 57
Cystine	56 ± 32	46 ± 10	47 ± 12	67 ± 16	49–53	36–61	55	Tr–52	44–50	34–40	53–58	51–54	46–63	48 ± 14	46 ± 18
Methionine	39 ± 6	38 ± 5	33 ± 6	41 ± 8	29–41	31–33	14–39	29–44	39–50	29–31	34–40	34–37	40–46	35 ± 7	32 ± 10
Isoleucine	99 ± 37	95 ± 6	92 ± 13	103 ± 8	82–90	79–83	42–48	78–113	78–99	52–65	73–85	73–94	99–105	90 ± 18	69 ± 19
Leucine	178 ± 27	179 ± 14	164 ± 34	179 ± 23	136–169	122–135	62–127	151–212	135–174	96–122	131–145	138–163	143–178	159 ± 35	133 ± 41
Tyrosine	76 ± 9	62 ± 5	55 ± 10	64 ± 25	56–65	52	Tr–47	62–87	57–74	53–65	73–85	71–83	76–82	66 ± 12	49 ± 10
Phenylalanine	75 ± 12	82 ± 10	63 ± 13	93 ± 10	62–80	55–57	Tr–65	73–91	68–79	50–62	73–82	63–88	78–90	75 ± 14	60 ± 11
Lysine	218 ± 28	243 ± 31	238 ± 45	238 ± 41	193–243	170–172	90–176	174–238	202–237	163–193	177–329	190–265	233–241	215 ± 52	201 ± 60
Histidine	99 ± 18	118 ± 9	90 ± 9	121 ± 12	97–115	78–81	49–91	81–113	103–121	67–87	86–91	88–118	84–103	101 ± 22	91 ± 20
Taurine	164 ± 38	184 ± 36	86 ± 42	207 ± 49	128–249	82–94	62–107	188–226	65–127	52–114	43–121	69–127	167–195	148 ± 46	148 ± 42
Aspartic acid	25 ± 3	40 ± 18	30 ± 30	31 ± 15	23–51	12–16	Tr–16	34–47	Tr–16	10–20	15–20	16–21	35–37	27 ± 10	23 ± 6
Serine	208 ± 48	229 ± 27	152 ± 64	235 ± 53	203–241	112–120	60–221	153–208	182–218	96–110	145–162	131–172	132–185	184 ± 60	176 ± 69
Glutamine + asparagine	505 ± 111	528 ± 129	336 ± 100	413 ± 190	561–692	317–514	187–676	203–535	573–637	329–512	368–526	361–655	243–319	398 ± 118	512 ± 232
Proline	168 ± 35	287 ± 44	167 ± 58	246 ± 13	249–293	104–143	111–276	166–292	148–247	122–170	157–201	182–239	175–202	211 ± 66	270 ± 74
Glutamic acid	178 ± 73	190 ± 100	190 ± 110	196 ± 110	99–200	103–120	97–101	147–304	138–265	169–306	223–310	179–349	270–502	243 ± 117	229 ± 145
Citrulline	32 ± 7	50 ± 13	34 ± 11	54 ± 5	51–53	24–26	Tr–42	23–38	31–37	26–29	31–37	34–39	Tr–21	37 ± 12	39 ± 30
Glycine	457 ± 56	406 ± 78	349 ± 119	476 ± 159	305–461	234–261	208–660	284–336	333–419	298–389	242–330	227–291	275–309	344 ± 73	474 ± 146
Alanine	671 ± 245	501 ± 48	532 ± 168	713 ± 52	574–704	380–572	278–703	476–635	493–593	476–547	441–496	478–745	540–568	575 ± 100	981 ± 140
Ornithine	81 ± 18	139 ± 17	114 ± 34	143 ± 13	150–155	55–60	42–104	113–144	68–79	45–70	59–85	75–108	97–123	109 ± 41	97 ± 37
Arginine	81 ± 7	82 ± 10	65 ± 21	102 ± 23	64–73	93–99	21–86	74–105	73–127	69–80	94–114	62–110	80–90	83 ± 19	78 ± 20

Values shown are mean and standard deviation, or range, micromoles per liter of serum.   [a] Number of subjects. Means of all values available for a given subject constitutes that individual's entry.

on more than one occasion. These results illustrate the high degree of variation that exists for all amino acids within a given subject. No single amino acid is outstandingly consistent, but deviations range from about 15% of the mean value (lysine, histidine) to 100% (aspartic acid). Variation in a single amino acid is, to some extent, idiosyncratic. For example, glycine varied considerably more in subject 0203 than in subject 0201. In the ranges given in Table 4, for two or three samples from a single subject, the high and low values were fairly equally distributed among the samples. Comparing these values with corresponding data in Table 4 for the entire population studied (n = 26), it is evident that the variation within an individual on the control diet is as great as the variation within the population of similarly treated subjects.

Differences in plasma amino acid patterns due to reduced dietary protein allowance are also seen in Table 4, even though variation is equally large among subjects fed a protein-free diet as the control diet. Generally, the concentrations of essential (and semi-essential) amino acids were decreased with the protein-free diet, while levels of non-essential amino acids followed no uniform directional change. Although these were not necessarily the most consistent, the greatest magnitudes of change in EAAs were in levels of threonine (-41%) and valine (-40%). Of the NEAA, alanine increased most (38%). Less than 20% difference was seen in 12 of the 20 amino acids. Data on fasting serum levels, available for 13 men given both control and protein-free diets, show that statistically significant differences were limited to seven amino acids: proline, methionine, and ornithine (p = .01); serine, citrulline, leucine, and phenylalanine (p = .05).

When subjects were fed the protein-free diet, in contrast to the control diet, the sum of EAA was lower but the sum of NEAA was about the same and, therefore, disproportionately higher. Ratios of some NEAA to EAA were calculated from these data (Table 4). For all subjects who received 12 g of egg white nitrogen, the fasting ratio was 1.06±0.24, while those fed the protein-free diet had a mean ratio of 1.60±.60. The ratios found in the 13 paried sets differed significantly due to diet (ratios 1.16 and 1.91, p < .001).

Table 5 shows that fasting serum amino acids did not vary according to length of time subjects had ingested a constant diet containing either 12 g of egg white nitrogen or essentially no protein. Duration of feeding prior to sampling blood ranged from one to 84 days for the control diet and from one to 18 days for the protein-free diet. Differences due to diet were as

Table5 17

Variation in Fasting Serum Free Amino Acids of Men Fed
a Constant Diet for Differing Lengths of Time (µM/liter)

Days of Feeding	n	Methionine	Valine	Alanine	Glycine
Control diet (12 g egg white N/day)					
1	3	23-39[a]	253-294	572-1030	261-660
3-4	7	36±61[a]	281±36	552±64	296±52
11-12	10	38±8	280±63	579±145	395±110
17-19	8	32±6	292±64	542±112	301±79
28-30	7	35±8	314±16	606±122	325±53
43-45	9	42±3	331±52	629±112	400±60
66	2	33-35	317-361	239-535	345-398
84	2	37-45	268-403	459-833	316-439
Protein-free diet					
1	4	27±7	172±37	537±231	303±25
6-8	3	33-33	143-167	146-1000	355-438
14-15	9	31±10	139±35	1047±370	430±103
17-18	2	29-36	130-270	346-1270	397-619

[a] Ranges of individual values are entered if n < 4; if n > 4, entries are mean ± standard deviation.

evident after one day of feeding as at later times. If there are continued changes in pattern with prolonged duration of deficient diet, these were obscured by inter-individual variation.

Serum amino acid levels were consistently found to be lower (-7 to -57%) in samples obtained 2-3 hrs after a protein-free meal than in conventional fasting samples. Statistically, differences in seven amino acids were significant in paired sets of data (p < .05, n = 6): methionine, leucine, phenylalanine, serine, citrulline, proline, and ornithine.

When the meal contained one-fourth of the day's protein allowance (3 g egg white nitrogen), three amino acids -- methionine, cystine, and arginine -- were unchanged from fasting levels but all others decreased. Meal-induced differences were significant for ten amino acids (leucine, isoleucine, serine, tyrosine, glycine, alanine, taurine, citrulline, ornithine, and aspartic acid). Thus, either there was no elevation of plasma amino acids due to protein feeding or it must have occurred earlier than one hour after a meal.

Our measurements show that, while a characteristic pattern of amino acids may be present with a given dietary nitrogen level, even under very closely controlled conditions intra- and inter-individual variation is about 15-25% of the mean concentration for many amino acids and as high as 100% for others. Such deviation may reflect transient events or characteristic individual differences in amino acid utilization. Some variation is doubtless methodologic.

Other investigators have reported that fasting plasma amino acids of chicks and rats are reduced by feeding a non-protein diet. Our data reveal a similar reduction in serum free amino acids of men due to ingestion of a protein-free meal. This reduction could be due to decreased tissue breakdown or to increased amino acid reutilization. In contrast to most published data, however, we found that serum amino acids were not elevated 1-2 hrs after a protein-containing meal. The values actually were lower, though not significantly so in all cases. Our conditions were quite different from those of other studies cited, in that our subjects were well conditioned to the diet and the test procedures, the test meal contained a balanced nutrient mixture and normal protein level, and the fasting patterns were determined after a period of constant ingestion of the exact same diet.

3. Characterization of Radioprotective Factor in Alfalfa

Attempts to process alfalfa fraction beyond the steps of cold-water extraction and freeze-drying have resulted in substantial loss of radio-protective (RP) activity. Earlier studies have shown RP factor to be sensitive to oxygen, and it is probable that oxidation occurs after the factor is separated from the antioxidants naturally present in alfalfa. Antioxidants that have RP activity for rats, BHT and propylgallate, were added to guinea pig diet to determine if these could be used as processing additives during isolation of RP factor. In contrast to the rat, guinea pigs did not tolerate the substances well. Mortality was greater after irradiation with 0.5% BHT in the diet than with the bran-oats diet alone. Addition of alfalfa both diminished radiation sensitivity and offset the detrimental effect of BHT.

Ash of whole alfalfa improves response to radiation but a mixture of Ca, Mg, Na, K, and Cl salts does not account for the entire effect. Ash prepared from the water-soluble RP fraction is not as effective as whole alfalfa ash. In irradiated animals, the effects of RP fraction and ash are additive, indicating that some mineral(s) is deficient or imbalanced in the basal diet and that RP factor is not an inorganic compound.