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9. ABSTRACT

Skim milk protein was replaced isonitorgenously by glycine and diammonium citrate, or by a mixture of non-essential amino acids in the test diet of 21 male college students; the effect of this replacement on urinary nitrogen and sulfur excretion and on fasting plasma amino acid concentration was studied. The subjects were fed a constant nitrogen intake equivalent to 0.38 g protein/kg body weight. Skim milk protein furnished 90% of the daily protein; oatmeal furnished the remainder. In experiments 1 and 2, glycine and diammonium citrate replaced 20 and 25% of total dietary nitrogen, respectively. In experiment 3, a mixture of non-essential amino acids replaced 25% of dietary nitrogen. The 20% replacement with glycine and diammonium citrate did not increase urinary nitrogen excretion in any of the seven subjects, but three of the seven showed lower urinary nitrogen excretion after return to the basal diet. A 25% replacement with glycine and diammonium citrate increased urinary nitrogen excretion in three of seven subjects and four subjects showed lower nitrogen output during the final basal period. The 25% replacement with non-essential amino acids did not change urinary nitrogen excretion but three of ten subjects showed a variable decrease in nitrogen excretion during the second basal period. As a replacement for 25% of total dietary protein, non-essential amino acids appeared a more effective non-specific nitrogen source than glycine and diammonium citrate. Fasting plasma amino acid levels were unchanged during the experimental periods.

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Partial Dietary Replacement of Milk Protein by Nonspecific Nitrogen in Young Men [1,2]

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ABSTRACT Skim milk protein was replaced isonitrogenously by glycine and diammonium citrate or by a mixture of nonessential amino acids in a series of [illegible] of 21 days each to study the effect of this replacement on urinary nitrogen and other excretion, nitrogen balance, plasma amino acid concentrations, and [illegible]. The subjects were fed a constant intake of milk protein equivalent to 0.55 g/kg/day. Skim milk was in formulated form of [illegible] protein; entire formula the remainder. In experiment 1 and 2, glycine and diammonium citrate replaced [illegible] of total dietary nitrogen, respectively. In experiment 3, a mixture of nonessential amino acids replaced 25% of dietary nitrogen. The 25% replacement with [illegible] monosodium citrate diet [illegible] nitrogen excretion in all of the [illegible] subjects, but [illegible] urinary nitrogen excretion [illegible] to the basal diet. A 25% replacement with glycine and diammonium citrate [illegible] urinary nitrogen excretion of [illegible] and four [illegible] subjects showed [illegible] excretion during the test period. A 25% replacement with [illegible] amino acids [illegible] urinary nitrogen excretion but 3 of [illegible] subjects showed [illegible] the diet [illegible] basal period. [illegible] 25% of total dietary protein nitrogen nonessential amino acids [illegible] a [illegible] increase in [illegible] diammonium citrate. Plasma amino acid levels were unchanged during the experimental periods.

Most of the work done on human protein requirements has dealt with the aspect of quantity and quality of the protein requirement, but the other component of the protein requirement, nonspecific nitrogen (1-4), which is furnished by excess essential amino acids and by the nonessential amino acids (NEAA), needs further investigation. A review of the published values for the essential amino acid requirements of adult man (2, 5, 6) suggests that the concentrations of essential amino acids per gram of total nitrogen in high quality protein foods such as eggs, meat and milk are higher than required when these foods are the sole source of dietary protein. Moreover, a number of investigators have shown in experimental animals that nonessential amino acids are more effective in meeting nonspecific nitrogen requirements than are the essential amino acids (7, 8). To investigate further the interrelationship between these two components of dietary protein needs, we have conducted three series of studies in which various amounts of the test dietary protein were replaced, isonitrogenously, by a source of nonspecific nitrogen. The present work with milk protein extends the previous observations [illegible] with egg (9) and lean protein (10).

In establishing the amino acid requirements of man, investigators have used various sources of supplemental nitrogen including glycine or urea (11), glycine in combination with diammonium citrate and other nonessential amino acids (12, 13), or a mixture of several nonessential amino acids (14). Although these studies suggest that a number of individual nonessential amino acids can meet the nonspecific nitrogen needs as effectively as a mixture of nonessential amino acids, the issue is far from settled (15). In the present series of experiments, we have used nitrogenously replaced milk protein [illegible]

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by a mixture of glycine and diammonium citrate, and then by a mixture of nonessential amino acids patterned as in cow's milk protein, and compared the effects.

MATERIALS AND METHODS

Subjects. The 21 male college students (table 1) who participated in these studies were in good health as determined by medical history and examination, and continued their normal daily activities throughout the experimental period. Three of the subjects (WI, KE and JR) participated in more than one experiment.

Diet. The composition of the diet, based principally on a liquid formula, is shown in table 2. Dried nonfat skim milk protein supplied 90% of the total dietary protein; oatmeal supplied the remainder. It was found necessary to include a small amount of oatmeal to maintain a stable formula of agreeable consistency. The diet furnished 0.38 g protein/kg per day. The net protein utilization (NPU) of the skim milk protein by rat assay (16) was determined to be 71 ± 2 compared with 63 ± 2 for unsupplemented casein used as a standard. The daily diet was divided into four approximately equal portions.

"Dilution" of the dietary protein. As in our previous studies (9, 10), the dietary nitrogen was replaced ("diluted") by substituting part of the dietary nitrogen with either a mixture of glycine and diammonium citrate (in which each compound supplied equal amounts of nitrogen) or a mixture of nonessential L-amino acids based upon the proportions of amino acids in whole dried nonfat skim milk protein (17). The composition of this mixture is shown in table 3.

Experimental sequence. The subjects were first fed the basal (0% dilution) diet which supplied 0.38 g protein/kg body weight. This rate was calculated to supply nitrogen at the minimum daily nitrogen requirement as discussed previously (9, 10). The duration of basal period I varied

TABLE 1 *

Age, height, weight and caloric intakes of young men studied for the effects of nonspecific nitrogen replacement of milk protein

Exp.	Subject	Age	Height	Body wt		Caloric Intake [1]
				Beginning	End	
		year	*cm*	*kg*	*kg*	*kcal/kg body wt*
1	WD	21	183	69.4	68.8	47
	WI	19	165	66.6	64.4	52
	DK	23	183	67.1	67.7	47
	CK	23	170	60.0	59.5	44
	RR	19	180	74.6	70.5	42
	LD	21	173	77.7	77.1	40
	RB	20	173	85.4	85.0	34
2	PD	19	181	72.1	71.2	51
	KE	21	180	76.4	76.9	44
	ND	19	162	61.3	62.6	42
	TJ	27	175	68.0	69.4	49
	JR	22	183	75.1	75.7	45
	MS	18	177	71.4	72.6	45
	RW	23	179	74.1	74.0	49
3	KE	21	180	82.4	83.5	42
	DH	20	167	84.2	84.1	42
	JL	19	181	78.5	78.9	42
	SM	22	163	71.0	71.2	45
	JR	22	183	77.7	78.4	41
	ER	19	177	77.7	77.4	46
	GS	23	185	88.1	88.8	50
	IS	20	187	65.6	67.5	48
	LD	22	173	77.1	75.9	42
	WI	19	165	69.1	67.5	50

[1] Calculated from food composition tables (Watt, B. K., and A. L. Merrill 1963 Composition of Foods. Agriculture Handbook 8, Washington, D.C.) and data supplied by the manufacturers of some of the dietary components.

from 14 to 18 days, depending upon the time required for the subject to reach an apparently steady state of nitrogen metabolism as revealed by urinary nitrogen excretion.

TABLE 2

Composition of diet given to young men studied for the effects of replacement of milk protein with nonspecific nitrogen

Ingredient	Amount per day [1,2]
	g
Nonfat dried skim milk [3]	65.0
Oatmeal [4]	110.8
Dexin [5]	130.0
Oil [6]	140.0
Vanilla	10.0
Lemon juice	5.0
NaCl	1.0
Avicel [7]	5.0
K_2HPO_4	3.5
H_2O	500.0

[1] Amount for a 70-kg subject. Intake for each subject adjusted according to body weight.

[2] In addition, cornstarch [illegible] (cornstarch, sugar and artificial [illegible]), and [illegible] beverages were given according to individual caloric needs. [illegible] tablet supplying [illegible] mg Fe, kindly supplied by [illegible], Kline & [illegible], Inc., Philadelphia, and a [illegible] (Upjohn Company, Kalamazoo, [illegible]) multivitamin capsule were given daily to all subjects. [illegible] capsule is stated to contain: [illegible] 1.5; vitamin D, [illegible]; vitamin [illegible] 2.5; [illegible] 2.5; vitamin C, [illegible]; nicotinamide, [illegible] 20; [illegible] thenate, [illegible] HCl, [illegible]. Subjects were also allowed Trident Gum (donated by American Chicle Company, New York). Five or six salt tablets, each supplying 1 g NaCl, were given to provide additional sodium, required.

[3] Carnation Company, Van Nuys, Calif.

[4] Dried, cooked Buckeye Homogenized Rolled Oats, kindly supplied by Quaker Oats Company, Barrington, Ill.

[5] Burroughs Wellcome and Co., Tuckahoe, N. Y. Approximate composition: dextrins 75%; maltose 24%.

[6] Corn oil, Mazola, Best Foods Division, Corn Products Company, New York.

[7] F.M.C. Corporation, Marcus Hook, Penna. Microcrystalline alpha cellulose.

TABLE 3

Composition of mixture of nonessential amino acids used to dilute milk protein

Amino acid	By wt
	%
L-Arginine-HCl	6.15
L-Histidine-HCl, H_2O	4.44
L-Alanine	5.80
L-Aspartic acid	12.28
L-Glutamic acid	39.26
Glycine	3.33
L-Proline	18.72
L-Serine	9.93
Total	100

Three experiments were conducted. The first two, with seven subjects each, evaluated the effects of replacing 20 and 25%, respectively, of dietary protein by a nonspecific nitrogen source, a mixture of glycine and diammonium citrate. Experiment 3, with 10 subjects, explored the effects of 25% isonitrogenous replacement of milk protein by a mixture of nonessential amino acids (table 3). The dilution period lasted for 20 or 21 days after which the subjects were returned to the basal diet for a period of 7 to 10 days. After basal period 2 the experiment was terminated.

Handling and analyses of samples. Urine was collected for complete 24-hour periods. Total dietary nitrogen, urinary nitrogen and creatinine were analyzed as described previously (9, 10). Urea nitrogen (10) and total sulfur (18) were measured on pooled urine samples and serum total protein determined (19). On the final day of each dietary period a blood sample was taken at 8 AM after an overnight fast (9 to 10 hours), and plasma free amino acids were measured by ion exchange chromatography.[8] The plasma was prepared for analysis as described by Mechanic et al. (20). Tryptophan was determined according to the procedure of Duggan and Lindenfriend (21). Results for urinary nitrogen were evaluated by the *t* test and by the paired *t* test for plasma amino acids.

RESULTS

Total urinary nitrogen excretion during the experimental periods of both the 20 and 25% replacement of milk protein by glycine and diammonium citrate is given in table 4. For the 20% replacement (exp. 1) no significant increase in nitrogen excretion was observed in any of the seven subjects at this dilution when compared with the initial basal period. Three subjects, however, showed a significantly lower urinary nitrogen excretion during the final basal period than during the initial basal period.

During the 25% dilution period (exp. 2), three of the seven subjects showed a significantly higher urinary nitrogen excretion compared with basal period 1, and most of the subjects showed a decreased

[8] Technicon Amino Acid Analyzer, Technicon Corporation, Ardsley, N. Y.

rate of urinary nitrogen excretion when they were returned to the basal diet at the end of the experiment (table 4). The increased nitrogen retention during this final period, as compared with the dilution period, was statistically significant ($P < 0.01$) in four of the seven subjects (table 4). The results suggested that the nitrogen of the diet diluted 20% with glycine and diammonium citrate was not as well utilized as that of the 20% diluted and basal diets. The decreased urinary nitrogen excretion during basal period 2 of the experiment compared with basal period 1 suggests that dilution of the milk protein may have resulted in a depletion of the body's pool of an essential amino acid(s) during the period of replacement by nonspecific nitrogen.

As summarized in table 5, urea excretion followed the same trends as total urinary nitrogen excretion during the experimental periods of experiments 1 and 2. Subjects fed a 20% dilution diet showed a higher rate of urea nitrogen excretion during the dilution period, and most of them showed a lower urea nitrogen excretion upon return to the undiluted diet. The results for total urinary sulfur excretion and the total urinary N/S ratio are also given in table 5. Total sulfur excretion was decreased by about 10% during the 25% dilution period compared with basal period 1, and for some subjects sulfur excretion tended to return to initial levels during the final period on the basal diet.

Subject DK showed a substantially higher total urinary sulfur excretion during the 20% dilution period (table 5), and a markedly decreased total sulfur excretion when returned to the undiluted diet despite opposite trends in total S-amino acid intake. This subject also showed a significant increase in nitrogen retention when returned to the basal diet during the final period of the study. There may have been an interaction between the nonspecific nitrogen source and the utilization of S-amino acids in this individual. Moreover, three of the four subjects in experiment 2 (PD, KE and MS) showing lower total urinary nitrogen output during basal period 2 also showed a decrease in total urinary sulfur output. These results suggest that above a certain level which varies with individuals, the dilution of milk protein with glycine and diammonium citrate as a nonspecific nitrogen source influences the utilization and metabolism of the sulfur amino acids which are limiting in this protein (1).

TABLE 4

Effect of dilution of milk protein with glycine and diammonium citrate on urinary nitrogen excretion (g/day) by young men (exps. 1 and 2)

Subject	Nitrogen intake	Dilution of dietary protein			
		0%	20%	25%	0%
	g/day				
WD	4.22	3.92 ± 0.65(6)[1]	4.01 ± 0.86(13)	--	4.14 ± 1.03(7)
WI	4.08	4.51 ± 0.38(5)	4.08 ± 0.52(11)**	--	3.70 ± 0.41(7)***
DK	4.09	4.25 ± 0.51(9)	4.11 ± 0.39(11)	--	3.59 ± 0.36(7)***
CK	3.36	2.69 ± 0.67(6)	2.89 ± 0.32(8)	--	2.93 ± 0.42(6)
RR	4.56	3.76 ± 0.41(7)	4.29 ± 0.60(9)	--	3.89 ± 0.52(6)
LD	4.80	5.56 ± 0.33(8)	5.50 ± 0.23(9)	--	5.09 ± 0.67(9)
RB	4.91	4.43 ± 0.41(7)	4.48 ± 0.43(8)	--	3.74 ± 0.33(9)***
PD	4.39	4.11 ± 0.46(5)		4.67 ± 0.30(9)**	4.02 ± 0.32(10)**
KE	4.65	4.42 ± 0.33(8)	--	4.51 ± 0.22(10)	3.95 ± 0.17(10)***
ND	3.73	3.44 ± 0.27(6)	--	3.52 ± 0.52(10)	3.19 ± 0.31(9)
TJ	4.11	3.49 ± 0.32(5)	--	3.67 ± 0.36(6)	3.37 ± 0.21(10)
JR	4.57	4.42 ± 0.47(5)	--	4.66 ± 0.33(9)**	4.16 ± 0.37(9)*
MS	4.35	3.41 ± 0.30(6)	--	4.27 ± 0.33(11)***	3.52 ± 0.[illegible](9)**
RW	4.51	5.07 ± 0.70(7)	--	4.88 ± 0.31(11)	4.65 ± 0.[illegible](10)

[1] Mean ± SD for number of observations shown in parentheses. Total lengths of dietary periods were 11 to 16 days and 7 to 16 days for the 0%, 20% or 25% and 0% dilution periods, respectively.

* Significantly different from basal period 1.

** Significantly different from dilution period.

* $P < 0.05$.

** $P < 0.01$.

TABLE 5

Effect of dilution of milk protein with nonspecific nitrogen on urinary urea, sulfur (g/day) and the total N/S ratio in young men [1]

Exp. no.	Subject	Urea nitrogen	Sulfur	N/S [2]	Urea nitrogen	Sulfur	N/S	Urea nitrogen	Sulfur	N/S
1				Dilution of protein by glycine and diammonium citrate						
			0%			20%			0%	
	WD	2.3	0.255	15.4	2.9	0.252	14.0	2.2	—	—
	WI	3.1	0.351	15.1	2.4	0.285	14.3	2.2	0.351	10.5
	DK	2.7	0.354	12.1	2.3	0.516	8.0	1.4	0.123	29.2
	Mean	2.7	0.319	13.5	2.5	0.364	12.1	1.9	0.237	19.9
2			0%			25%			0%	
	PD	2.8	0.357	11.5	3.1	0.424	14.4	2.2	0.264	15.2
	KE	2.9	[illegible]	12.7	3.0	0.318	14.2	2.3	0.281	12.8
	ND	2.2	[illegible]	14.0	2.4	0.198	17.8	1.9	0.216	11.8
	TI	2.7	[illegible]	10.8	2.3	0.246	11.9	2.0	0.240	14.0
	JR	2.9	[illegible]	12.4	3.5	0.273	17.1	2.5	0.297	14.9
	MS	1.9	[illegible]	13.6	2.4	[illegible]	16.9	2.2	0.221	15.9
	RW	3.0	0.359	13.7	3.5	0.342	14.3	3.5	0.363	12.8
	Mean	2.6	0.345	12.7	3.0	0.279	15.7	2.4	0.260	14.2
	SD	0.4	0.055	1.2	0.5	0.051	1.5	0.5	0.050	1.2
3				Dilution of protein by nonessential amino acids						
			0%			25%			0%	
	LE	2.6	0.327	13.1	3.0	0.235	15.6	2.6	0.321	11.9
	DH	3.6	[illegible]	12.3	2.9	0.242	12.8	3.6	0.384	11.5
	JE	3.0	[illegible]	11.2	2.9	0.232	15.2	2.7	0.239	11.5
	SM	2.5	[illegible]	13.9	2.6	0.216	17.3	2.6	0.274	13.9
	JR	2.7	[illegible]	14.0	3.0	0.306	13.7	2.6	0.297	12.8
	LR	2.5	[illegible]	12.4	3.0	0.261	15.9	3.1	0.297	14.1
	GS	2.4	[illegible]	14.9	2.9	0.273	16.3	2.1	0.318	11.6
	IS	1.5	[illegible]	8.4	3.2	0.536	12.8	3.5	0.313	14.3
	LD	3.2	[illegible]	14.4	3.3	0.364	12.6	3.3	0.375	12.3
	WI	2.6	[illegible]	14.5	2.5	0.336	14.5	2.5	0.341	14.3
	Mean	2.6	[illegible]	12.4	2.9	0.295	14.4	2.9	[illegible]	12.5
	SD	0.6	[illegible]	1.6	0.3	0.042	1.9	0.5	0.033	1.1

[1] Analyses on pooled urine samples for periods shown in tables 4 and 6.
[2] Ratio of total urinary nitrogen to total urinary sulfur. Values for total nitrogen taken from tables 4 and 6.

The results given in tables 4 and 5 show that total nitrogen and urea nitrogen output increased during a 25% dilution with glycine and diammonium citrate and decreased to a variable degree when subjects were returned to the basal diet. The question remained as to whether the glycine and diammonium citrate mixture was the most effective source of nonspecific nitrogen. Accordingly, a mixture of nonessential amino acids (table 3) was tested at the 25% level of dietary protein dilution. In this experiment (table 6 and [illegible]) of the 10 subjects showed a statistically significant decrease in urinary nitrogen output following return to the basal diet after the 3-week period of 25% dilution with the NEAA mixture. Three subjects showed a lower urinary nitrogen excretion during basal period 2 as compared with the initial basal period. Subject KE previously showed a similar response following a 25% dilution of the dietary nitrogen with glycine and diammonium citrate (table 4).

Urinary nitrogen output during the dilution period did not increase significantly above that of the initial basal period, except in the case of two subjects. In one case (IS), however, a relatively low rate of excretion was observed during basal period 1, and during the final basal period urinary nitrogen output was the same as that during the dilution period. The low initial basal level of nitrogen excretion was probably atypical but the reason is unknown.

The results for urea nitrogen and total urinary sulfur excretion for the 10 subjects given nonessential amino acids as the source of nonspecific nitrogen are sum-

TABLE 6

Effects of dilution of milk protein with a mixture of nonessential amino acids on urinary nitrogen excretion by young men (exp. 3)

Subject	Nitrogen intake	Dilution of dietary protein		
		0%	25%	0%
	g/day	*g N/day*	*g N/day*	*g N/day*
KE	5.01	4.30±0.20(8) [1]	4.46±0.21(8)	3.81±0.32(9) [a] [illegible]
DH	5.12	5.22±0.58(8)	4.38±0.43(5) [a] [b]	4.40±0.44(7) [a] [b]
JL	4.77	4.42±0.33(7)	4.28±0.33(9)	4.01±0.[illegible](9)
SM	4.32	3.93±0.36(10)	3.74±0.38(10)	3.85±0.[illegible](9)
JR	4.73	4.25±0.20(6)	4.19±0.33(9)	3.80±0.[illegible](7) [a] [illegible]
ER	4.73	3.77±0.96(10)	4.14±0.46(9)	4.19±0.[illegible](9)
CS	5.38	4.38±0.67(12)	4.45±0.80(9)	3.70±0.[illegible](9)
IS	3.99	2.78±0.10(8)	4.29±0.55(9) [a] [b]	4.55±0.[illegible](9) [a] [b]
LD	4.77	4.70±0.45(6)	4.56±0.66(8)	4.61±0.[illegible](8)
WI	4.16	4.04±0.62(5)	3.27±0.47(6)	3.85±0.[illegible](7)

[1] Mean ± SD for number of observations shown in parentheses. Total length of 0, 25 and 0% dietary periods were 16 to 18, 20 to 21 and 9 days, respectively.
[a] Significantly different from basal period 1.
[b] Significantly different from dilution period.
[c] $P < 0.05$.
[d] $P < 0.01$.

marized in table 5. During the dilution period, total sulfur excretion was lower than during the initial basal period. The mean decrease in total urinary sulfur excretion was slightly greater during this period than in the corresponding period of experiment 2 involving use of glycine and diammonium citrate.

In contrast to results obtained in experiment 2 (table 5), total urinary sulfur excretion did not appear to decrease further during basal period 2 for KE, DH and JR. These three subjects showed increased nitrogen retention in the final basal period as compared with the initial basal period. In experiment 2 with glycine and diammonium citrate, a lower sulfur output was observed during basal period 2 for the three subjects (PD, KE and MS) who showed increased nitrogen retention after the 20-day period of dilution. These results suggest that the mixture of NEAA may be a slightly more effective source of nonspecific nitrogen for the isonitrogenous replacement of skim milk protein than glycine and diammonium citrate.

Serum total protein and the concentrations of free amino acids determined for subjects studied at a 25% level of replacement with the two sources of nonspecific nitrogen are summarized in table 7. No significant differences ($P > 0.05$) in the concentrations of these blood constituents were detected. During dilution with nonessential amino acids (exp. 3) only alanine increased, but because of high variability this change was not statistically significant.

DISCUSSION

The results of urinary nitrogen excretion in the present experiments indicate that for most human subjects, milk protein, when fed at about the minimum requirement level (0.38 g protein/kg per day), can be replaced with 20% of nitrogen from nonspecific nitrogen sources without affecting its nutritive value. The extent of replacement possible varied with the subjects from about 20% to at least 25%; this may reflect variability in the requirement for individual essential amino acids (5, 6). The influence of dilution appeared to be partially affected by the source of nitrogen used for the replacement of milk protein nitrogen, because glycine and diammonium citrate resulted in a greater increase in urinary nitrogen excretion during the 25% dilution period than the isonitrogenous mixture of nonessential amino acids.

Kofranyi and Jekat (22) in their study with adult subjects concluded that only 10 to 15% of milk protein could be replaced with ammonium citrate without a change in the value of the protein. The difference between their results and ours may be due to their limited number of subjects and use of ammonium citrate alone as the nonspecific nitrogen source. Clark et al.

TABLE 7

Serum total protein and free amino acids in fasting plasma of young men studied for effects of dilution of milk protein with nonspecific nitrogen

Amino acid	Dilution of protein					
	0%	25%	0%	0%	25%	0%
	Nonessential amino acids			Glycine and diammonium citrate		
Essential						
Threonine	186 ± 24[1]	166 ± 42	174 ± 33	201 ± 31	211 ± 26	181 ± 26
Valine	198 ± 25	219 ± 61	210 ± 26	205 ± 29	174 ± 32	182 ± 40
Isoleucine	76 ± 14	88 ± 27	77 ± 7	86 ± 12	65 ± 6	63 ± 12
Leucine	121 ± 15	146 ± 44	131 ± 18	134 ± 25	115 ± 20	112 ± 23
Tyrosine	62 ± 9	68 ± 21	71 ± 10	77 ± 22	57 ± 11	55 ± 13
Phenylalanine	63 ± 7	67 ± 19	62 ± 5	64 ± 14	55 ± 8	55 ± 11
Lysine	187 ± 36	222 ± 75	201 ± 27	195 ± 28	161 ± 21	179 ± 29
Tryptophan	65 ± 8	72 ± 12	74 ± 9	61 ± 7	57 ± 10	63 ± 12
Nonessential						
Aspartic	67 ± 13	65 ± 15	52 ± 13	70 ± 10	70 ± 8	64 ± 7
Serine and glutamine	180 ± 52	232 ± 119	159 ± 66	149 ± 68	198 ± 22	169 ± 20
Glutamic	313 ± 38	334 ± 37	312 ± 73	341 ± 50	309 ± 55	289 ± 40
Glycine	307 ± 59	398 ± 123	294 ± 25	335 ± 25	401 ± 51	312 ± 56
Alanine	666 ± 136	809 ± 289	643 ± 188	557 ± 115	490 ± 127	501 ± 118
Histidine	120 ± 10	122 ± 30	117 ± 26	117 ± 21	92 ± 12	100 ± 4
Arginine	99 ± 32	130 ± 24	105 ± 20	121 ± 47	91 ± 19	74 ± 8
Serum protein (g/100 ml)	7.1 ± 0.4	7.2 ± 0.2	7.3 ± 0.4	7.0 ± 0.3	6.9 ± 0.3	7.0 ± 0.3

[1]Micromoles per liter. Mean ± SD for samples taken at 8:00 AM after a 9 to 10 hour fast at the end of each period.

(13) observed in young adults given 9.0 g nitrogen/day (of which approximately half was provided by the supplementary nonspecific nitrogen) that nitrogen retention was depressed when diammonium citrate was fed, but not when glycine alone was given. Although none of the seven young adults in the present study appeared to retain less nitrogen when 20% of milk protein was replaced with the glycine and diammonium citrate mixture, three appeared to show additional retention when returned to the basal diet. This latter observation may mean that an amino acid depletion had occurred which was not apparent from measuring urinary nitrogen excretion alone during the dilution period. This possibility was not reflected, however, by a reduction in the concentration of essential amino acids in fasting plasma samples. The results suggested that milk protein can be replaced to a greater extent in most young adults than indicated by the study of Kofranyi and Jekat (22).

Snyderman et al. (2) studied infants from 3 weeks to 5 months of age and found that weight gain and nitrogen balance could be maintained despite a partial substitution of evaporated cow's milk protein by glycine or urea. Although their experiments were not designed to determine the extent to which milk protein nitrogen could be replaced by nonessential nitrogen, it appears from their data that 20% of cow's milk protein could be isonitrogenously replaced by either glycine or urea without reducing infant growth. This is probably an overestimate, however, since the level of protein intake in their study was above minimum requirements for growth (23).

In establishing amino acid requirements of man, investigators have used different sources of supplemental nitrogen (11–14). Swendseid et al. (24) have reported that glycine alone is not well used by man as a nonspecific nitrogen source; however, they observed that a combination of glycine and diammonium citrate was as effective a source of nonspecific nitrogen as a mixture of nonessential amino acids. Experiments with weanling rats have shown that different sources of nonspecific nitrogen can affect growth rate in the presence of minimal amounts of essential amino acids (25, 26). Swendseid et al. (27) have

shown that the growth rate of rats may be depressed by a low protein diet supplemented with 7.5% glycine. The results of our study with young men suggest that there may be some difference in the utilization of glycine and diammonium citrate, compared with a mixture of nonessential amino acids, when included as nonspecific nitrogen supplements in a diet supplying nitrogen at approximately the minimum requirement.

In experimental animals glycine alleviates the growth-depressing effect of a high methionine intake (28, 29) and conversely, methionine protects against excess glycine (30). Sulfur amino acids are limiting in cow's milk protein and therefore, under conditions of a relatively low intake of these amino acids in the present studies, a comparison of the effects of glycine-diammonium citrate and a mixture of nonessential amino acids on urinary nitrogen excretion was considered appropriate. Although a close relationship between urinary nitrogen and sulfur excretion has been noted by a number of investigators (31, 32), a difference in the pattern of excretion between these elements has also been reported (33). Our results with subjects who reacted to a 20 or 25% dilution with glycine-diammonium citrate by showing changes in urinary sulfur excretion suggest that this nitrogen source may affect S-amino acid metabolism at the level of protein intake used in this study. Further studies are required to evaluate more fully the significance of these observations.

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