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Research Article

THE ORAL AND PARENTERAL PHENYLALANINE REQUIREMENTS FOR NITROGEN EQUILIBRIUM IN MAN[1]

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From published data on the minimum quantity of whole protein (milk, soy flour, white flour, and egg) required (1) or estimated (2) to maintain nitrogen balance in adults, and from analytical data of the amino acid content of these proteins (3), Harte and Travers (4) have calculated the "minimum" requirements for man of the essential amino acids. For phenylalanine, 1.4 Gm. daily was considered to be sufficient. The observations reported here indicate that the daily phenylalanine requirement for man is greater when the daily protein intake (protein hydrolysate) is given intravenously than when given orally. These studies were made during the course of investigations employing as a sole source of nitrogen a 10 per cent solution of amino acids[2] prepared by complete acid hydrolysis of casein, largely freed of glutamic and aspartic acids, and supplemented with dl-tryptophane, dl-methionine, and glycine (5, 6).

MATERIALS AND METHODS

In Table I the composition of two lots of the solution of amino acids with respect to the 8 amino acids essential for man (7) and for arginine and histidine is shown and is compared to that of casein. The results are based on analyses by microbiological assay (8, 9, 10). Likewise the quantity of amino acids in 1,000 cc. of this 10 per cent solution (average of the two nearly identical lots) is compared with the "minimum" amino acid requirements calculated by Harte and Travers (4). It is apparent that from 2 to 15 times these "minimum" quantities of amino acids are supplied in 1,000 cc. of the solution with the exception of phenylalanine, which might therefore be expected to be the "limiting" amino acid in this solution. The low phenylalanine content of these particular lots (1.7 per cent and 1.9 per cent) enabled us to investigate the effect on the nitrogen balance in normal human subjects of this "limiting" essential amino acid. The solution of amino acids employed now regularly contains at least 4 per cent of l-phenylalanine.

A basal diet essentially devoid of protein (supplying 0.1 to 0.5 Gm. nitrogen daily) but adequate in calories, vitamins, and salts was used in all studies. Its composition has been described previously (11). All amino acid analyses were by microbiological assay (8). Since the unnatural isomer of phenylalanine is neither readily utilized by man (12) nor detected by microbiological assay (8), all values presented here are for the naturally occurring l-form. The daily urine and pooled stool nitrogen analyses were determined by the standard micro- or macro-Kjeldahl methods. The alpha amino nitrogen was determined by the gasometric ninhydrin method as described by Hamilton and Van Slyke for plasma (13), and by Van Slyke, MacFadyen and Hamilton for urine (14).

TABLE I

Comparison of the composition of the solution of amino acids with casein, and the quantity of amino acids in 1,000 cc. of the 10 per cent solution with the "minimum" amino acid requirements for man

Amino acid	Amino acid solution* (l-form) Gm. per liter of 10 per cent solution		Casein† Gm. per 100 Gm.			"Minimum" amino acid requirement for man‡	Amino acid solution (Average of lots A and B) 1,000 cc., 10 per cent
	Lot A	Lot B	a	b	c		
						grams per day	*grams per day*
Arginine	5.9	5.2	3.9	3.6	3.7	1.2	5.6
Histidine	2.2	1.9	2.8	2.6	3.0	0.5	2.1
Isoleucine	6.0	6.2	5.6	6.4	8.6	1.2	6.1
Leucine	12.2	11.5	9.9	9.9	10.5	1.7	11.9
Lysine	13.0	10.8	7.7	8.3	6.7	0.8	11.9
Methionine	5.8	5.4	2.6	2.6	3.1	0.5	5.6
Phenylalanine	1.7	1.9	5.9	5.2	4.8	1.4	1.8
Threonine	1.9	1.9	4.2	4.2	4.6	1.0	1.9
Tryptophane	0.9	0.8	1.1	1.2	1.4	0.4	0.9
Valine	5.5	6.0	6.7	6.2	5.8	1.1	5.8

* Also contains 0.9 Gm. d-tryptophane, 2.5 Gm. d-methionine, and 22.6 Gm. glycine per liter of 10 per cent solution. Microbiological assay.
† a—Stokes *et al.* (8).
b—Hodson and Krueger (9).
c—Pearce *et al.* (10).
‡ Harte and Travers (4).

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[2] Developed and supplied by Merck and Company, Inc., Rahway, New Jersey.

The basal diet was ingested at regular meal hours. The solution of amino acids, when administered intravenously, was injected in 1 to 2 hours once daily without added glucose, and was preceded and followed by food by mouth so that approximately 1,000 calories were given within 1 hour before and 2 hours after the infusion. When the intravenously injected phenylalanine-poor solution of amino acids was supplemented by phenylalanine by mouth, the oral phenylalanine (except in one instance mentioned later) was given approximately 1 to 2 hours prior to the intravenous infusion. The solution of amino acids, when administered orally, was ingested in several divided feedings per day together with the basal protein-free diet.

RESULTS

In Table II are recorded the nitrogen and phenylalanine intake and excretion of 3 normal adult males, each of whom received orally or intravenously 1,000 cc. of the solution of amino acids daily as the sole source of nitrogen. As will be noted (Table II and Figure 1), subject P. C. was in nitrogen equilibrium during an initial period while receiving, in addition to the solution of amino acids intravenously, an orally administered supplement of phenylalanine. Together, these supplied a total of from 2.7 to 2.9 Gm. of phenylalanine daily. When oral phenylalanine supplementation was omitted in the second period, reducing the intake of this amino acid to 1.9 Gm., a negative nitrogen balance promptly resulted. However, subsequent administration during a third period of the amino acid solution orally in several feedings daily for 8 days without the phenylalanine supplement (phenylalanine intake 1.9 Gm. daily) resulted in nitrogen equilibrium except for days 8 and 12. Subjects E. B. and M. D. O. (Table II), who were both given the amino acid solution by rapid intravenous infusion once daily, likewise substantiate the finding that 1.9 Gm. of phenylalanine when given by the intravenous route did not maintain nitrogen equilib-

TABLE II

Nitrogen and phenylalanine intake and excretion in 3 subjects given 1,000 cc. of the 10 per cent solution of amino acids daily

Subject	Period	Day	Nitrogen total daily intake *grams per day*		Nitrogen total daily output *grams per day*		Nitrogen balance	l-Phenylalanine total daily intake *grams per day*		l-Phenylalanine total daily output	Administered l-phenylalanine excreted in urine
			i.v.	Oral	Urine	Stool		i.v.	Oral		
							grams per day			*mgm. per day urine*	*per cent*
P. C.	I	1	15.7	0.5	13.4	1.0	+1.8	1.7	1.0	57.7	2.1
		2	15.7	0.5	12.1	1.0	+3.1	1.7	1.0		
		3	14.3	0.5	14.6	1.0	−0.8	1.9	1.0		
		4	14.3	0.5	13.7	1.0	+0.1	1.9	1.0	189.0	6.5
		5	14.3	0.5	13.6	1.0	+0.2	1.9	1.0		
	II	6	14.3	0.4	15.3	1.0	−1.6	1.9	0	218.0	11.5
		7	14.3	0.4	15.7	1.0	−2.0	1.9	0		
	III	8	0	14.7	17.5	0.9	−3.6	0	1.9		
		9	0	14.7	13.5	0.9	+0.3	0	1.9		
		10	0	14.7	13.6	0.9	+0.2	0	1.9	32.9	1.7
		11	0	14.7	13.7	0.9	+0.1	0	1.9		
		12	0	14.7	15.2	0.9	−1.4	0	1.9		
		13	0	14.7	13.6	0.9	+0.2	0	1.9		
		14	0	14.7	13.4	0.9	+0.4	0	1.9	32.7	1.7
		15	0	14.7	13.1	0.9	+0.7	0	1.9		
E. B.	I	1	15.7	0.5	15.4	0.5	+0.3	1.7	1.0	130.0	4.8
		2	15.7	0.5	15.9	0.5	−0.2	1.7	1.0		
M. D. O.	I	1	13.7	0.1	14.5	0.2	−0.9	1.8	0		
		2	14.3	0.1	12.5	0.2	+1.7	1.9	0	200.0	10.5
		3	14.3	0.1	15.9	0.2	−1.7	1.9	0		
		4	14.3	0.1	15.0	0.2	−0.8	1.9	0		
		5	14.3	0.1	16.6	0.2	−2.4	1.9	0	221.0	11.6
		6	14.3	0.1	15.2	0.2	−1.0	1.9	0		
		7	14.3	0.1	15.4	0.2	−1.2	1.9	0		

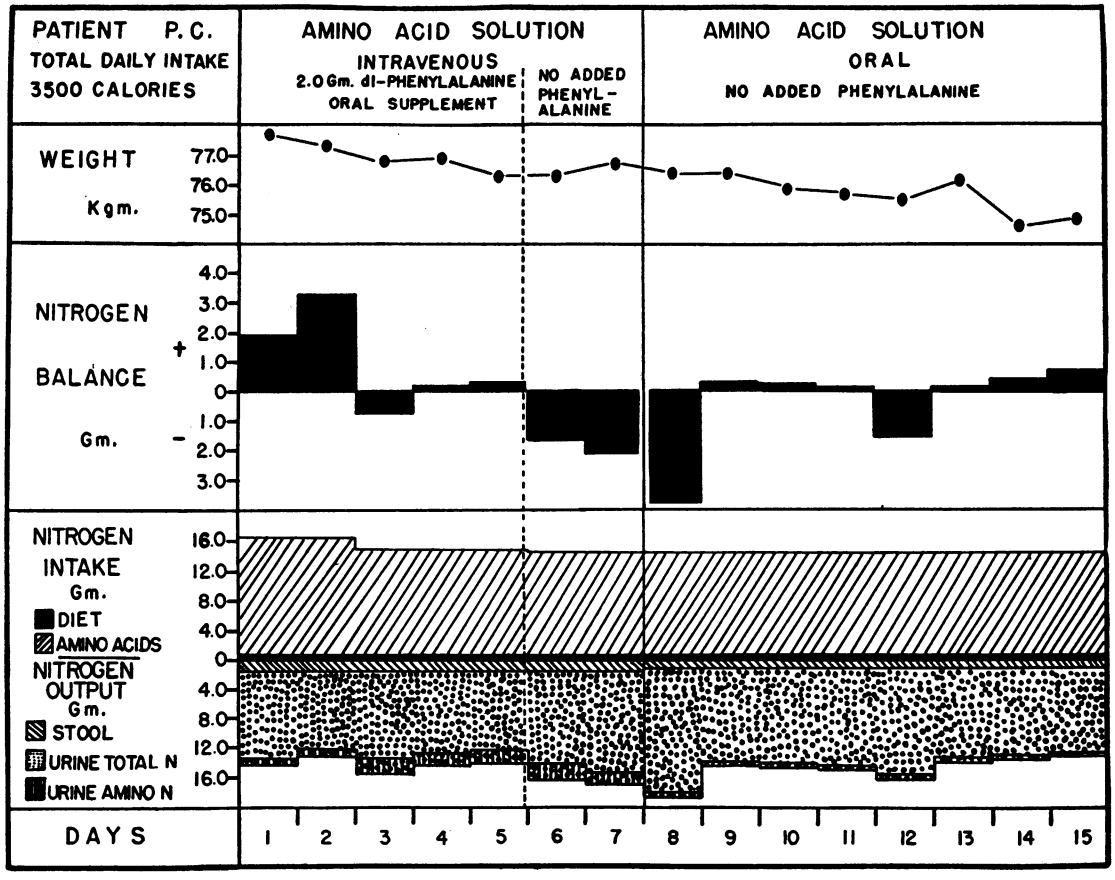

FIG. 1. NITROGEN BALANCE OF PATIENT P. C. WHILE RECEIVING AMINO ACID SOLUTION INTRAVENOUSLY, THEN ORALLY

rium until additional phenylalanine was provided.

Subjects P. C. and E. B. who were in nitrogen equilibrium while receiving the parenteral amino acid solution supplemented with phenylalanine given by mouth excreted 58 mgm. and 189 mgm. (P. C.) and 130 mgm. (E. B.) of microbiologically available free phenylalanine in the urine daily. These amounts represent 2.1 per cent, 6.5 per cent, and 4.8 per cent, respectively, of the administered l-phenylalanine. Subjects P. C. and M. D. O., while receiving the intravenous amino acid solution without additional phenylalanine, were in negative nitrogen balance, P. C. excreting 218 mgm., and M. D. O. excreting 200 mgm. and 221 mgm. of free urinary phenylalanine. This represented an increase to 11.5 per cent, 10.5 per cent, and 11.6 per cent, respectively, of that administered. While in nitrogen equilibrium on the unsupplemented low-phenylalanine amino acid solution administered orally, subject P. C. excreted only 33 mgm. of phenylalanine daily, or 1.7 per cent of the dosage fed.

DISCUSSION

In this laboratory the average phenylalanine excretion in normal adult males on an *ad libitum* feeding is 14 mgm. daily (range 7 to 28 mgm. for 8 individuals [15]). One individual who maintained weight and nitrogen balance on 80 Gm. of whole ("Labco") casein daily as the sole source of nitrogen excreted 0.5 per cent of the ingested phenylalanine (5.0 Gm. fed and 24 mgm. excreted daily [15]). Others (16 to 18) also have found but minute quantities (3 to 10 mgm.) excreted daily by normal individuals. In contrast is the somewhat greater absolute and percentage excretion of phenylalanine when the solution of amino acids was ingested orally by subject P. C.

TABLE III

Urine alpha amino nitrogen excretion in normal subjects given whole casein orally, the amino acid solution orally, and the amino acid solution intravenously

Subject	Protein source	Route of administration	Alpha amino nitrogen administered	Alpha amino nitrogen excreted in urine *mgm. per 4-hour period*			
				Control fasting	After administration	Net urinary loss	
			mgm.			*mgm.*	*per cent*
R. D. E.	Whole casein	Oral	5,440	28.7	52.1	23.4	0.4
C. S. D.	Whole casein	Oral	5,440	26.4	43.3	16.9	0.3
R. D. E.	Amino acid solution	Oral	5,440	28.7	181.3	152.6	2.8
C. S. D.	Amino acid solution	Oral	5,440	26.4	189.2	162.8	3.0
8 Normal adult males (average)	Amino acid solution	Intravenous	6,000	17.2	581.5	564.3	9.4

Since West, Wilson, and Eyles (19) found higher blood levels of amino nitrogen in infants who had ingested an acid hydrolysate of casein than after ingestion of an equivalent amount of whole protein (casein), we felt that a higher blood level might account for the increased excretion of phenylalanine in subject P. C. Two of the present authors (R. D. E. and C. S. D.), fasting on two successive days, ingested equivalent amounts of nitrogen first as whole casein and on the second day as the amino acid solution. The blood amino nitrogen values obtained hourly post-ingestion were variable for both subjects and were not markedly different after the ingestion of either protein, but both subjects excreted several times greater quantities of amino nitrogen in the urine (4 hours post-ingestion) after the amino acid ingestion than after the casein feeding. In Table III and Figure 2 are presented data on the absolute and percentage excretion of amino nitrogen following the administration of equivalent amounts of nitrogen as whole casein orally, as the amino acid solution orally, and as the amino acid solution intravenously (20). The average net urinary amino nitrogen loss during the first 4 hours [3] after the orally administered whole casein was 20.1 mgm. (0.4 per cent), after the orally ingested amino acid

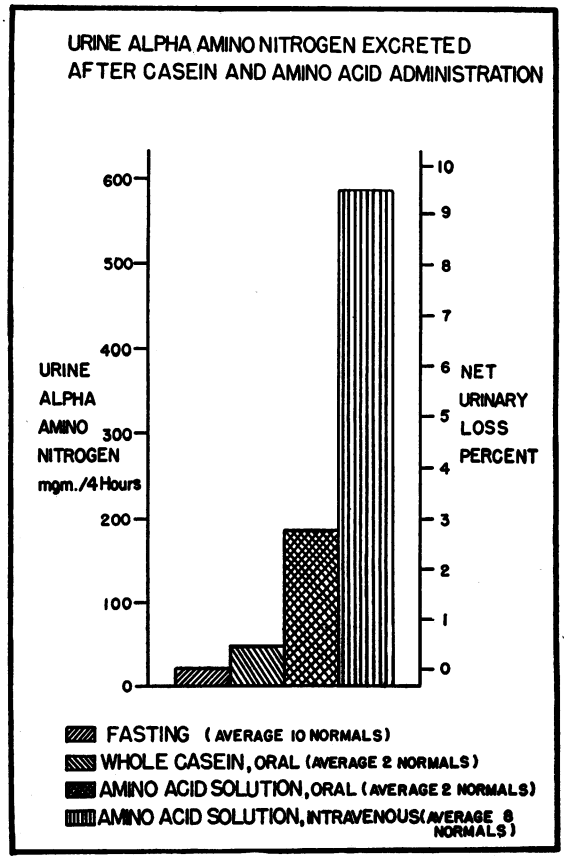

FIG. 2

[3] A 4-hour period of urine collections was chosen since the loss of amino nitrogen in the urine due to the infusion of the solution of amino acids was complete within this period, by which time the elevated blood amino nitrogen value had returned to normal (20). However, since the elevated blood amino acid values following whole protein and amino acids by mouth do not return entirely to normal by this time (19), apparently reflecting digestion and absorption, a longer collection period would have been more desirable. Nevertheless, it is our experience that the subsequent loss of amino nitrogen is so small it would not detract from the significance of these observations.

solution was 157.7 mgm. (2.9 per cent), and after the intravenously injected amino acid solution was 564.3 mgm. (9.4 per cent). Thus, although the finding in subject P. C. of increased urinary phenylalanine excretion when the amino acid solution rather than whole protein was given by mouth could not be definitely related to high blood amino nitrogen values, it was associated with an increased excretion of amino nitrogen, including all the amino acids fed, and was not due to the selective excretion of phenylalanine.

When the solution of amino acids was administered parenterally to the subjects reported here, they excreted 4 to 16 times the quantity of phenylalanine normally found in the urine, presumably because of the marked rise in blood amino acid values following the rapid intravenous infusion of the amino acid solution. The absolute quantity of phenylalanine excreted after parenteral infusion was actually slightly less when 2.0 Gm. of dl-phenylalanine was added as an oral supplement than when none was given. Furthermore the percentage excretion of the phenylalanine administered was from 2 to 5 times greater with no supplementation than when supplemental phenylalanine was given orally. This would suggest that at least part of the supplemental phenylalanine was conserved or retained when sufficient was administered to allow nitrogen equilibrium, while when less than the minimum required for nitrogen equilibrium was available, a portion of that given was wasted. Similar observations were made by Pearce, Sauberlich and Baumann (10) who found that mice fed incomplete proteins excreted a much greater percentage of the ingested amino acids in the urine in microbiologically available form than did mice fed complete proteins.

When a protein hydrolysate or mixture of amino acids deficient in one or more essential amino acids is given in order to maintain nitrogen equilibrium, the limiting amino acid(s) must be so administered that all the essential amino acids are present in the body at approximately the same time (21). Recent studies have suggested that the maximum time interval may be as short as one hour (22). The slight negative nitrogen balance of subject P. C. on day 3 may be interpreted in this way, for he received the oral phenylalanine supplement one-half hour after the infusion had been administered; or, of course, it may merely be a chance observation. At other times the supplemental phenylalanine was administered orally 1 to 2 hours prior to the infusion, for maximum blood levels (measured microbiologically) have been shown to occur after such an interval following ingestion (23). A more ideal procedure would have been to administer this amino acid parenterally with the phenylalanine-deficient solution of amino acids. The intravenous injections of the amino acid solution were preceded and followed by approximately 1,000 food calories by mouth in order to achieve maximum retention of the nitrogen (24).

The smaller quantity of phenylalanine required daily for maintenance of nitrogen balance when the solution of amino acids was given orally rather than intravenously may have been due to more efficient and economical utilization with divided oral feedings than with the single rapid daily intravenous injection. Further, the greatly increased urinary excretion of free phenylalanine and other amino acids following the intravenous infusions as compared to that following the oral feedings must have decreased the quantity of free phenylalanine available for metabolic purposes. That 1.9 Gm. of phenylalanine daily does not necessarily represent the "minimum" oral requirement is acknowledged. The smaller calculated value of Harte and Travers (4) of 1.4 Gm. daily may be nearer the true minimum. Likewise, 2.7 Gm. of phenylalanine may be greater than the minimum required for parenteral administration, but certainly under the conditions of our observations, 1.9 Gm. daily did not suffice. It must be borne in mind that man can probably partially utilize the unnatural isomer of phenylalanine (12). Thus, when we gave 2.0 Gm. of dl-phenylalanine as an oral supplement, more than 1.0 Gm. (the l-form) may have been nutritionally available. Minimum oral requirements for amino acids will undoubtedly be found insufficient when the conditions of the study are altered. Thus the quantity required may be expected to be greater when hydrolyzed rather than when whole protein is administered orally, and still greater when parenteral alimentation is used.

CONCLUSIONS

1. A solution of amino acids containing 1.9 Gm. of l-phenylalanine will maintain nitrogen balance

in normal adult man if given orally in several divided feedings daily.

2. The same solution of amino acids is not capable of maintaining nitrogen balance if given parenterally in one rapid injection daily unless additional phenylalanine is simultaneously provided. Under these conditions, 2.7 Gm. of l-phenylalanine will maintain nitrogen balance.

3. The urinary loss of phenylalanine was greater both in absolute and in percentage amounts of that administered when less than the minimum requirement of the amino acid was given and negative nitrogen balance resulted, than when sufficient of the amino acid was available for nitrogen equilibrium.

4. The oral administration of the amino acid solution resulted in an 8 times greater urinary loss of amino acids than when whole protein was fed. The parenteral administration of the amino acid solution resulted in a 28-fold increase.

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