THE AMINO ACID REQUIREMENTS OF MAN

III. THE RÔLE OF ISOLEUCINE: ADDITIONAL EVIDENCE CONCERNING HISTIDINE*

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Earlier papers of this series (1, 2) have demonstrated (*a*) that the ten amino acids previously found to be dispensable dietary components for the growing rat (3) and the adult dog (4) are also dispensable for man, (*b*) that valine, methionine, and threonine are essential constituents of the food of human subjects, and (*c*) that histidine, contrary to expectations, is not necessary for the maintenance of nitrogen balance, nor for the preservation of a sense of physical well being in adults. The absence of objective and subjective symptoms following the exclusion of histidine from the diet is in striking contrast to the sequelae observed after valine, methionine, or threonine deprivation. A deficiency of one of the latter amino acids is followed by a pronounced negative nitrogen balance, a marked failure in appetite, a sensation of extreme fatigue, and a distinct increase in nervous irritability. These symptoms manifest themselves even when the subjects are unaware that a dietary alteration has been made, and disappear when the missing amino acid is returned to the food.

The present paper is a continuation of the previous studies, and is concerned primarily with the dietary significance of isoleucine. Incidental to the main objective, additional evidence is presented regarding the dispensability of histidine in the species in question.

EXPERIMENTAL

Elsewhere (1) are described the general procedures employed in the conduct of the experiments, the methods used in analyzing the foods and excreta, and the techniques involved in the preparation of the amino acid

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solutions, the wafers, and the other components of the diets. The composition of the wafers and the amino acid mixtures varied from one experiment to another, depending upon the purposes at hand and the caloric requirements of the subjects. All other conditions, including the make-up of the baking powder which served as a leavening agent for the wafers and the daily intakes of inorganic salts and vitamins (*cf.* (1), Tables I to III), were kept constant throughout the entire series of investigations.

The composition and daily intake of the amino acid mixture (Mixture A-1) used in the studies herein described are shown in Table I. The ratio of its components is identical with that present in Mixture A, which was employed in the valine and methionine experiments (1). However, for reasons which are unrelated to the dietary rôle of isoleucine, and therefore need not be discussed here, the intake of each amino acid was slightly higher than in the previous investigation, and furnished a total of 7.66 gm. of nitrogen daily instead of 6.70 gm. Furthermore, the isoleucine tests were initiated before the status of histidine had been established; consequently Mixture A-1 carried the latter amino acid. Four racemic acids were present in the mixture. Three of these, namely valine, isoleucine, and threonine, were doubled in amount over the desired levels on the supposition that the D isomers might not be utilized by man. On the other hand, no such allowance was made in the case of DL-methionine. Since the two optical isomers of this amino acid are equally effective in the rat (5), the assumption was made tentatively that a like situation might exist in the human species. Of the 7.66 gm. of nitrogen in Mixture A-1, 1.37 gm. were derived from the D forms of valine, isoleucine, and threonine. Thus, as has been the practice in all of our human experiments, the intake of nitrogen of known effectiveness was quite low.

The subjects received a large part of their calories in the form of wafers. Table II records the composition and daily intake of Wafers II. The unidentified nitrogen present in the daily allotment amounted to 0.30 gm., and was derived almost entirely from the starch. Additional calories, as needed by the subjects, were supplied in the form of sucrose and purified butter fat (1). Generally, the sucrose was used as a flavoring agent for the amino acid solutions. The butter fat served as a spread for the wafers. Cellu flour, in appropriate amounts (*cf.* (1) pp. 545–546), was consumed by the subjects as a water suspension rather than as a component of the wafers.

Two healthy young men, 25 and 24 years of age, served as the subjects of the isoleucine experiments. At the beginning of the tests, subject D. T. W. weighed 71.3 kilos and subject A. B. 71.8 kilos. The caloric requirements of D. T. W. were met by the daily ingestion of Wafers II, 140 gm. of sucrose, and 95 gm. of butter fat. The diet of A. B. was identical, except that it contained only 80 gm. of butter fat over and above that present in the wafers. The diets were consumed in three equal parts each day. The total daily energy intakes of the two young men were

TABLE I

Composition of Amino Acid Mixture A-1

Component	Daily intake		
	Physiologically active	As used	N content
	gm.	*gm.*	*gm.*
Valine	4.89	9.78*	1.17
Leucine	5.61	5.61	0.60
Isoleucine	3.52	7.04*	0.75
Methionine	4.21	4.21*	0.39
Threonine	3.52	7.04*	0.82
Phenylalanine	4.90	4.90	0.41
Tryptophan	2.12	2.12	0.29
Lysine	7.02		
" monohydrochloride		8.78	1.35
Histidine	2.81		
" monohydrochloride monohydrate		3.80	0.76
Arginine	3.48		
" monohydrochloride		4.21	1.12
	42.08	57.49	7.66†

* Racemic acids.

† Of the 7.66 gm. of nitrogen contributed by Mixture A-1, 1.37 gm. were derived from the D isomers of valine, isoleucine, and threonine.

TABLE II

*Composition and Daily Intake of Wafers II**

Component	Daily intake	Approximate calories†
	gm.	
Corn-starch	356.00	1332
Sucrose	35.00	138
Butter fat (melted and centrifuged)	67.00	620
Corn oil	5.00	47
Salt mixture	4.71	
Baking powder (starch)	7.60	10
Sodium chloride	10.00	
	485.31	2147

* The daily intake of Wafers II contained 0.30 gm. of unknown nitrogen.

† After correcting for moisture.

3810 and 3670 calories[1] respectively. In each subject, the total nitrogen intake was 8.08 gm. daily, and the unidentified nitrogen from all sources (wafers, extra butter fat, liver concentrate used as a supplementary source of vitamins, and lemon juice (1)) amounted to 0.42 gm. daily.

The nitrogen balance data obtained in the two experiments are summarized graphically in Fig. 1. As will be observed, the fore period, during which the subjects ingested the complete diets, was 6 days in duration.

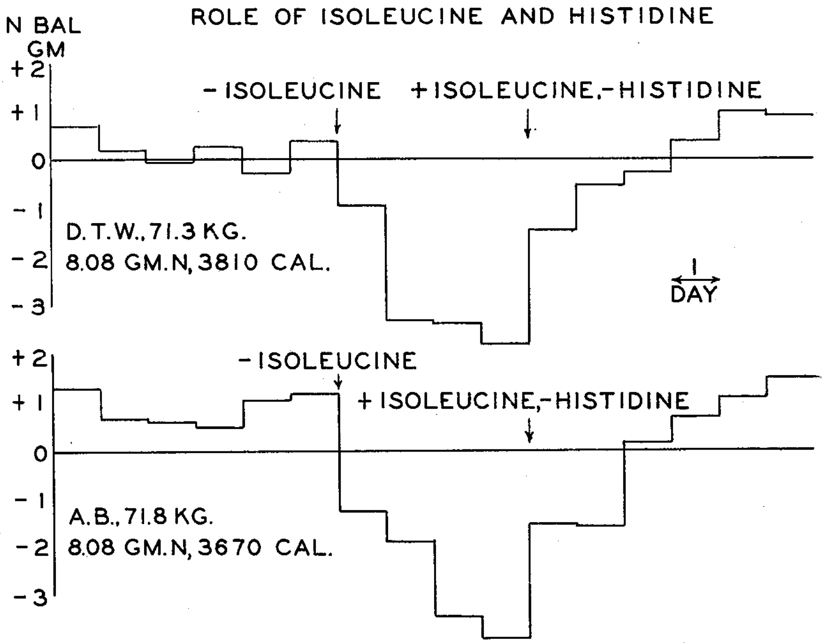

FIG. 1. The rôle of isoleucine and histidine in the maintenance of nitrogen equilibrium in man.

During that time, the nitrogen balance of D. T. W. manifested moderate fluctuations such as are encountered frequently in experiments of this nature, even when the rations contain proteins of superior quality in adequate amounts. His average daily balance for the period was +0.21 gm. Throughout the same period, A. B. experienced a fairly strong nitrogen retention, with an average daily balance of +0.90 gm.

At the expiration of the 6th day, isoleucine was removed from the food and the other amino acids were increased sufficiently to maintain a con-

[1] Attention has been called repeatedly (6, 1, 2) to the high caloric requirements of subjects receiving amino acid diets. Proof of this fact will be presented in a subsequent paper.

stant total nitrogen intake. The effects were immediate and dramatic. On the next day the subjects showed balances of −0.93 and −1.26 gm., respectively, and their nutritive status became worse as the deficiency was prolonged. On the 4th day of isoleucine deprivation, the output of nitrogen exceeded the intake by 3.79 and 3.90 gm. respectively. Both young men complained bitterly of a complete loss of appetite. Each experienced great difficulty in consuming his meals. Furthermore, the symptoms of nervousness, exhaustion, and dizziness, which are encountered to a greater or lesser extent whenever human subjects are deprived of an essential amino acid, were exaggerated to a degree not observed before or since in other types of dietary deficiencies. It became evident by the end of the 4th day that the subjects were approaching the limit of their endurance, and that the missing amino acid must be returned to the food without further delay.

This critical stage in the isoleucine tests was attained at a time when the authors were struggling with the problem of the dietary rôle of histidine in man. Despite evidence accumulated in several carefully controlled experiments, pointing definitely to the conclusion that this amino acid is non-essential for purposes of nitrogen equilibrium, difficulty was experienced by us in accepting the findings at face value (*cf.* (2)). This skepticism arose from the fact that histidine is an indispensable component of the food of all other species of mammals thus far tested. The isoleucine experiments then under way appeared to afford appropriate conditions for obtaining additional information regarding the nutritive importance of histidine. If the latter, it was reasoned, plays a significant rôle in nitrogen equilibrium, subjects experiencing the profound deficiency induced by the absence of isoleucine should not be expected to return to normal following the restoration of the missing amino acid, *provided simultaneously* histidine was withdrawn from the food. The status of D. T. W. and A. B. presented an opportunity, which was seized, of putting this hypothesis to the test. At the end of the 4th day of isoleucine deprivation, the latter amino acid was returned to the diets and all histidine was removed. The total nitrogen content was maintained at a constant level by suitable adjustments in the quantities of the other amino acids.

The effect of these alterations upon the nitrogen balance of the subjects is shown in Fig. 1, and is scarcely less remarkable than that induced by the exclusion of isoleucine. Immediately, the magnitude of the negative nitrogen balance diminished markedly. On the 3rd day, A. B. showed a slight positive balance, and this steadily increased in amount during succeeding days. D. T. W. attained a state of positive balance on the 4th day, and reached higher levels of retention during the next 2 days. Furthermore, all subjective symptoms diminished promptly in both subjects, and disappeared completely before the tests were discontinued at the expiration of the 6th day of isoleucine administration. Obviously, the unavailability of dietary histidine failed completely to preclude recovery from both the metabolic and psychological manifestations of the isoleucine deficiency.

In Table III is shown the distribution of nitrogen between the urine and feces of A. B. Entirely comparable data were obtained in the experiment

TABLE III

Rôle of Isoleucine and Histidine in Nitrogen Balance

Subject A. B., daily nitrogen intake, 8.08 gm.

Period	Body weight	N output		N balance	Blood			Diet
		Urine	Feces		Non-protein N	Plasma proteins	Hemoglobin	
days	*kg.*	*gm.*	*gm.*	*gm.*	*mg. per cent*	*gm. per cent*	*gm. per cent*	
1	71.8	5.60	1.11	+1.37				Complete; 10 amino acids
2	71.8	6.32	1.11	+0.65				
3	71.8	6.33	1.11	+0.64				
4	71.8	6.43	1.11	+0.54				
5	71.8	5.94	1.11	+1.03				
6	71.8	5.82	1.11	+1.15	28	7.0	16.7	
7	70.8	8.38	0.96	−1.26				No isoleucine; 9 amino acids
8	70.8	9.00	0.96	−1.88				
9	70.4	10.53	0.96	−3.41				
10	70.4	11.02	0.96	−3.90	32	7.5	16.8	
11	71.3	8.52	1.00	−1.44				No histidine; 9 amino acids
12	71.8	8.54	1.00	−1.46				
13	72.7	6.94	1.00	+0.14				
14	72.7	6.37	1.00	+0.71				
15	72.7	6.04	1.00	+1.04				
16	72.7	5.60	1.00	+1.48	28	6.8	15.9	

upon D. T. W., but are omitted to conserve space. Attention is called to the sharp rise in the output of urinary nitrogen following the removal of isoleucine from the food. On the 10th day of the experiment (4th day of isoleucine deprivation) it attained a level which was almost twice that observed on the 1st day. Also to be noted is the fact that, after isoleucine had been restored to the food, the absence of histidine did not prevent the urinary nitrogen from decreasing rapidly to its original level.

Analyses of blood samples taken at the end of each period showed that the whole blood non-protein nitrogen, total plasma proteins, and hemoglobin remained within normal limits (Table III). Though the results are not included in the tabular data, erythrocyte and leucocyte counts also were perfectly normal throughout.

In Table IV is summarized for A. B. the distribution of nitrogen between the several urinary components. All analyses were made at daily intervals, but the results are presented as period averages. The nitrogen distribution is, for the most part, just as one would expect with a normal diet of relatively low nitrogen content. An exception is the ammonia nitrogen, which increased from a moderate value during the fore period to a rather high one during the period of isoleucine deficiency, and failed to diminish appreciably during the after period. A like behavior of the ammonia nitrogen was observed in D. T. W. No explanation is available for this phenomenon. In both subjects the output of α-amino nitrogen was high. This is observed regularly under the conditions of our experiments and doubtless is attributable to the partial excretion of the D isomers of valine, isoleucine, and threonine. As indicated above, the daily intake of α-amino nitrogen in these forms during the fore period amounted to 1.37 gm.

TABLE IV

Urinary Nitrogen Partition (Period Averages)

Subject A. B., initial body weight, 71.8 kilos.

Period	Total N	Urea N	Ammonia N	Creatinine N	Creatine N	Uric acid N	α-Amino N	Rest N	Diet
days	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
6	6.07	3.28	0.77	0.61	0.03	0.10	1.04	0.24	Complete
4	9.73	6.52	1.01	0.62	0.05	0.15	0.97	0.41	No isoleucine
6	7.00	3.78	0.99	0.60	0.06	0.11	1.09	0.37	" histidine

The two experiments outlined above demonstrate unmistakably that *isoleucine is an indispensable component of the human diet.* As measured by the magnitude of the negative nitrogen balance and the intensity of the subjective symptoms, no other type of amino acid deficiency produces in man so prompt and so profound a nutritive failure. On the contrary, the ability of subjects upon an isoleucine-deficient diet to recover promptly when this amino acid is returned to the diet, despite the absence of histidine from the food, provides additional evidence in support of the conclusion (2) that *preformed histidine is not necessary for the maintenance of nitrogen equilibrium in adult man.* In view of the latter fact, all subsequent investigations of this series will involve the use of histidine-free diets.

SUMMARY

Further experiments designed to establish the amino acid requirements of man have demonstrated that *isoleucine is an indispensable dietary com-*

ponent. Its exclusion from the food is followed promptly by a negative nitrogen balance of greater magnitude than that encountered in any other type of amino acid deficiency. On the other hand, the removal of histidine from the diet of subjects who are experiencing an isoleucine deficiency does not prevent the reestablishment of positive nitrogen balance when isoleucine is restored to the food. This is interpreted as providing additional evidence in support of the conclusion (2) that *preformed histidine is not necessary for the maintenance of nitrogen equilibrium in adult man.*

BIBLIOGRAPHY

1. Rose, W. C., Johnson, J. E., and Haines, W. J., *J. Biol. Chem.*, **182,** 541 (1950).
2. Rose, W. C., Haines, W. J., Warner, D. T., and Johnson, J. E., *J. Biol. Chem.*, **188,** 49 (1951).
3. Rose, W. C., Oesterling, M. J., and Womack, M., *J. Biol. Chem.*, **176,** 753 (1948).
4. Rose, W. C., and Rice, E. E., *Science*, **90,** 186 (1939).
5. Wretlind, K. A. J., and Rose, W. C., *J. Biol. Chem.*, **187,** 697 (1950).
6. Rose, W. C., *Federation Proc.*, **8,** 546 (1949).