Influence of Time of Ingestion of Essential Amino Acids upon Maintenance of Nitrogen Balance.* (17508)

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Accumulating evidence supports the assumption that all of the essential amino acids must be available in the diet simultaneously for growth or weight gain.(1-4) Previous experiments from this laboratory(3) demonstrated that protein-depleted rats did not gain weight when 2 incomplete essential amino acid mixtures (which when mixed constituted a complete mixture) were offered in rotation even an hour apart. However, because of the abrupt loss of appetite resulting from feeding rations deficient in one or more essential amino acids,(5) it was difficult to ascertain how much of the recorded loss of body weight was due to inanition and how much to failure of supplementation of one deficient mixture by the other. Similarly poor food consumption may have influenced the results of others who have reported similar experiments in the weanling rat. (1,2) In view of the theoretical and practical importance of this problem to man, whose appetite apparently is not reduced so greatly by the absence of a complete mixture of the essential amino acids, the present experiment was performed. It was planned so that the alternate ingestion of 2 incomplete essential amino acid mixtures, which together contained all 9 amino acids essential for growth and maintenance of the rat,(5-8) could be accomplished while all food constituents were being supplied at an adequate level.

Plan. Two groups of 6 adult male Sprague-Dawley rats of comparable weight and age were force fed all of their food at 12 hour intervals for 14 days. For 7 days the first group received alternately ration 5A containing 5 of the 9 essential amino acids and ration 4A containing the other 4 essential amino acids. During this period the second group received at each feeding a complete ration

TABLE I.
Amino Acid Composition of Mixtures Force Fed Normal Rats.

Amino acids	MAA	4A	5A
	g per 100 g of dry ration		
DL Isoleucine	.66	1.32	—
L (+) Lysine HCl	.16	.32	—
DL Phenylalanine	.15	.31	—
DL Tryptophane	.06	.11	—
L (+) Histidine HCl	.08	—	.15
L (+) Leucine	.21	—	.41
DL Methionine	.19	—	.37
DL Threonine	.27	—	.55
DL Valine	.51	—	1.03
Total essential	2.29	2.06	2.51
DL Alanine	.58	.58	.58
L (+) Arginine HCl	.51	.51	.51
DL Aspartic acid	.65	.65	.65
L (—) Cystine	.04	.04	.04
L (+) Glutamic acid	2.44	2.44	2.44
Glycine	.05	.05	.05
L (—) Tyrosine	.66	.66	.66
Total non-essential	4.93	4.93	4.93

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TABLE II.
Nitrogen Balance and Weight Changes in Rats Force Fed Incomplete Amino Acid Rations Alternately.

Animal No.	Wt, g	1st 7 days Diet	1st 7 days Wt gain or loss, g	1st 7 days N bal., mg	2nd 7 days Diet	2nd 7 days Wt gain or loss, g	2nd 7 days N bal., mg
1	325	4A,	—13	—303	MAA	— 1	+ 37
2	328	5A	—13	—214	every	— 2	+ 83
3	306	alternated	— 7	—328	12	— 1	+ 15
4	313	every	—15	—226	hr	— 2	+ 85
5	305	12	— 9	—264		+10	+ 65
6	322	hr	—15	—276		+ 5	+ 91
Avg	317		—12	—269		+ 2	+ 63
7	332	MAA	— 8	+ 33	4A,	—10	—285
8	319	every	— 2	+ 50	5A	—12	—225
9	322		— 2	+ 7	alternated	—13	—316
10	333	12	± 0	+ 41	every	—17	—241
11	317	hr	— 2	+ 9	12	—12	—201
12	308		— 1	+ 71	hr	—13	—301
Avg	322		— 3	+ 35		—13	—262

(MAA) containing the 9 essential amino acids. The rations were prepared so that all of the rats would receive equal quantities of all amino acids as well as the other food constituents every 24 hours, the only difference being the time of ingestion of the essential amino acids. During the second 7 day period the rations were interchanged so that the first group received the complete mixture and the second group received the alternating incomplete rations. Nitrogen balances and weight changes were measured for each 7 day period.

Rations, method of feeding and nitrogen balances. The quantities of amino acids in the 3 rations are shown in Table I. The quantities in the complete ration (MAA) were derived from data recently obtained in this laboratory.(9) They represent the minimum quantities of each essential amino acid necessary just to maintain weight equilibrium when fed at the daily level of approximately 3.6 g of amino acids (1.15 g essential and 2.45 nonessential) per kg of body weight and about 1200 calories per M^2 of surface area. The concentrations of essential amino acids in each of the other rations (5A and 4A) were double those in MAA in order to make the total daily intakes equal. The remainder of each of the rations in the "dry" form was made up of dextrin 64%, corn oil 4%, ruffex 5%, salt mixture 4%, water 16% and vitamins in quantities previously reported.(5) Each of the "dry" rations was diluted with additional water to make a thin soup which was thoroughly mixed in a Waring Blendor and fed in 13 ml quantities each feeding. This quantity supplied about 3.84 g of amino acids per kg and 1280 calories per M^2 of surface area per 24 hours. The rations were force fed by the method of Shay and Gruenstein.(10) The rats were adapted to this method of alimentation during a preliminary 6 day period by force feeding gradually increasing quantities of the MAA ration to each rat. Nitrogen balances were determined by methods previously described.(8) Fecal and urinary nitrogens were determined separately. Nitrogen intake was estimated by means of Kjeldahl determinations on multiple aliquots of the diluted rations.

Results. A brief summary of the experimental results is presented in Table II. The ingestion of the two incomplete amino acid mixtures at 12 hour intervals led to a weight loss of about 2 g per day per rat and an average negative nitrogen balance of approxi-

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10. Shay, H., and Gruenstein, M., *J. Lab. and Clin. Med.*, 1946, v166, 429.

mately 260 mg for the 7 day period. On the other hand the rats receiving the complete ration each feeding almost maintained their weights and showed slightly positive nitrogen balances. This latter finding confirms the conclusion that the MAA ration is barely adequate to maintain nitrogen balance and weight when fed at the calorie level of $1200/M^2$. The results were essentially similar in each period. Thus the two sets of data gained by interchanging the diets after 7 days act as mutual checks and minimize the influence of the factors of time and animal variation. The fecal nitrogen values were not appreciably altered by the divided feeding of the essential amino acids. Furthermore, the total nitrogen ingested proved to be quite similar in the 2 groups during the 2 periods varying from 944 to 998 mg per rat for each 7 days. Therefore, the increased urinary nitrogen excreted by the rats receiving the essential amino acids in two feedings of incomplete mixtures at 12 hour intervals accounted almost entirely for the negative nitrogen balance which these animals exhibited. It is interesting to note that the difference between the average 7 day urinary nitrogens of the rats receiving the incomplete rations in rotation and those receiving the complete mixture each feeding was 359 mg. This is slightly greater than the total essential amino nitrogen of 316 mg/week which each rat received.

Discussion. These results suggest that the normal adult rat is unable to store even temporarily an incomplete mixture of amino acids for later use. Nor does there appear to be a means of drawing upon the abundant labile protein reserves(11) for a certain specific amino acid or certain amino acids which are absent from the diet. These data, therefore, confirm those reported for the protein-depleted rat(3) and suggest the rat's loss of appetite was probably a good indication of a lack of supplementation of the one deficient ration by the other. The only other nitrogen balance determinations done under conditions of delayed supplementation of which we are aware are those of Elman(12) who gave dogs tryptophane-deficient hydrolysates intravenously followed 6 hr later by tryptophane intravenously. Under these circumstances he noted a negative nitrogen balance. His experiments were not influenced by the speed of intestinal absorption or any problem of differential absorption of amino acids. Therefore it is of interest that the same type of result is obtained by oral force feeding in the rat if the stomach is allowed to empty before the supplementary ration is given. This inability of the normal adult animal to make up for an essential amino acid deficient even for a short time makes it even more important to avoid oral or parenteral feedings made up principally of incomplete proteins or amino acids especially during disease or convalescence. Further studies are needed to determine the source of the excess urinary nitrogen which is excreted under these circumstances.

Summary. The normal adult rat is unable to derive adequate nutritional value from an incomplete essential amino acid mixture which is supplemented 12 hours later by the missing amino acids even when attention is paid to strict equalization of the intakes of all food constituents.

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