Effect of 'Amino-acid Imbalance' on Growth and Vitamin A Storage in the White Rat

In the course of the studies concerning the interrelationship of leucine and norleucine[1,2,3] it was observed that rats fed low protein diets supplemented with norleucine or norleucine and leucine were retarded. When the animals were killed and their livers analyzed for vitamin A content, it was found that these animals invariably stored a lower amount of vitamin A when compared with the control group of animals (Table 1). In view of the fact that the inhibition of growth was always associated with a depressed appetite, it was of considerable interest to determine whether the low liver vitamin A values were a consequence of the reduced food intake and thus low vitamin A consumption or whether they resulted from apparent 'amino-acid imbalance'. In order to determine this a study was made in which the vitamin A intake was controlled.

Female weanling albino rats of the Sprague-Dawley strain were fed the appropriate diets, as indicated in Table 2, *ad libitum*, for a period of 3 weeks. At the end of this period the animals were killed and their livers analyzed for vitamin A content by the method of Ames *et al*[4]. The basal diet free of vitamin A contained 9 per cent casein (vitamin-free), 15 per cent glucose, 54 per cent sucrose, 2 per cent corn oil, 14 per cent hydrogenated-vegetable oil (Crisco), 2 per cent cellulose (Solka-Floc), 4 per cent salts (Jones-Foster mixture) and vitamins as described previously[5]. The added amino-acids replaced an equivalent amount of sucrose. Vitamin A was administered every other day at a level of 40 I.U. per rat.

The results of these experiments, presented in Table 2, show that the supplementation of 9 per cent casein diet with leucine and/or norleucine did not lower the storage of hepatic vitamin A as was anticipated. Instead there was a definite increase in the vitamin A content in the livers of rats receiving amino-acid supplements. The contrary observation made previously can thus be safely explained on the basis of different food intake.

The growth data are just the opposite from the vitamin A values. Norleucine markedly suppressed growth of rats especially when fed together with leucine. Supplementation of diet with leucine alone, however, resulted only in a slight growth depression. The data are conclusive in demonstrating the inverse relationship between the vitamin A content of the liver and the body weight gain.

The storage of vitamin A in the kidneys, also shown in Table 2, was found to be small when compared with the liver. It should be noted, however, that the inclusion of the excess amino-acids in the diet consistently lowered the kidney vitamin A.

Since the liver and the kidneys are the principal sites of the storage of vitamin A in the mammalian organisms, the sum of the vitamin A values in these organs, after correction for the initial content, should give the 'total' storage of vitamin A in the body. Similarly the amount of vitamin A which is lost or unaccounted for, as calculated by subtracting the vitamin A stored from the amount of vitamin A which was administered, can serve as a measure of the vitamin utilization. When such calculations are made it is evident that the more rapidly growing animals utilize more and store less vitamin A than do the slow growing animals. This hypothesis would account for the lower level of hepatic vitamin A in the rats fed casein when compared with similar diet supplemented with the excess amino-acids. This thesis is further supported by our recent observation[5] that the oral administration of equal amounts of vitamin A to growing rats resulted in a greater hepatic vitamin A storage in rats fed protein-free diet than in those which were kept on casein diet.

It is apparent from this as well as previously reported[5] studies that rats are able to store ample amount of vitamin A in the liver despite the amino-acid imbalance or in the complete absence of the dietary protein when the intake of vitamin A is controlled. Nevertheless the lack of or imbalance in the dietary protein may result in a lower storage of vitamin A simply because under these conditions the animals usually lose appetite which inevitably will result in lowered consumption of vitamin A.

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Table 1. Growth, Food Consumption and Vitamin A Storage in the White Rat Fed Imbalanced Diets

Group	No. of rats	Body weight gain (gm.)	Food intake (gm.)	Liver weight (gm.)	Liver vit. A (μgm.)	Kidney weight (gm.)	Kidney vit. A (μgm.)	Total vit. A (μgm.)
Nitrogen-free	2	−20	96	1·25	89·1	0·38	2·8	91·8
9% Casein	4	58	229	4·13	572·6	0·74	8·6	581·2
9% Casein+2% DL-norleucine	4	47	213	3·51	514·9	0·80	7·3	522·2
9% Casein+2% L-leucine+2% DL-norleucine	4	25	156	2·53	350·0	0·66	5·9	355·9

Table 2. Growth, Food Consumption and Vitamin A Storage in the White Rat Fed Imbalanced Diets under the Conditions of Equalized Intake of Vitamin A

	Control rats	Supplement to 9% Casein Diet: None	2% L-Leucine	2% DL-Norleucine	2% L-Leucine and 2% DL-Norleucine
Number of rats	2	3	3	3	3
Weight gain (gm.)	—	51	43	29	24
Food intake (gm.)	—	253	236	198	178
Vitamin A fed (μgm.)	—	126·0	126·0	126·0	126·0
Liver vitamin A (μgm.)	15·7	45·4	58·3	65·0	64·2
Kidney vitamin A (μgm.)	1·16	2·03	1·58	1·76	1·32
Total vitamin A stored (μgm.)	—	30·6	43·0	49·9	48·7
Vitamin A utilized (μgm.)	—	95·4	83·0	76·1	77·3