PLASMA PROTEIN PRODUCTION INFLUENCED BY AMINO ACID MIXTURES AND LACK OF ESSENTIAL AMINO ACIDS

A DEFICIENCY STATE RELATED TO UNKNOWN FACTORS*

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INTRODUCTION

This paper continues our study of the normal and abnormal physiology of the essential amino acids, in particular as they are related to the production of new plasma proteins. Depletion of the normal levels of plasma proteins in the circulating blood by means of plasmapheresis puts a strain upon the body and accentuates the normal demands for plasma protein production. It stimulates vigorous new plasma protein production perhaps at times to the point of diversion of protein-building materials from the tissues.

The observations of this report show that certain amino acids must be provided in the food intake for long continued plasma protein production and that other unknown dietary factors must be present to maintain normal function of the plasma protein-producing mechanisms. The experiments were not designed to study vitamin deficiencies but the synthetic diets necessary to the investigation provided certain unanticipated dietary deficiencies. In view of previous observations (5, 7) and of control observations in the present experiments afforded by casein feeding, we believe that the deficiencies evidenced by skin lesions and possibly associated with liver function impairment (reduced excretion of bilirubin) are not the result of amino acid inadequacies or imbalances. Other possible explanations are discussed. The effects of leucine, leucine and isoleucine, tryptophane, and histidine deficiencies are each recorded.

Methods

The general procedures of these experiments have been previously described or referred to (7). The amino acid mixtures were prepared as before and are listed in Table 1. Subcutaneous and intravenous infusions varied from 6 to 10 per cent amino acids in distilled water. Proper mixtures of amino acids in 10 per cent concentration are well tolerated subcutaneously.

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The basal diets are given in the experimental history of each dog. Vitamin supplements varied and the amounts given are recorded in the experimental histories. Liver powder (Eli Lilly and Company, fraction rich in B_2 complex), yeast powder (Standard Brands, Inc., No. 200-B), and two vitamin emulsions specially prepared by Eli Lilly and Company and Mr. A. L. Caldwell were used. Emulsion MH43-2231 contained per 10 cc. vitamin A 5000 u.s.p. units and vitamin D 500 units, and in milligrams, thiamin chloride 6, riboflavin 6, pyridoxin hydrochloride 5, calcium pantothenate 5, nicotinamide 50, ascorbic acid 50, mixed natural tocopherols 50, choline chloride 100, rice polish concentrate 1000. MH43-8111 contained in addition to the above 2-methyl-1,4-naphthoquinone-1, inositol 50, p-aminobenzoic acid 50, linoleic acid esters 500, and an increase in choline chloride to 300 mg.

TABLE 1

Amino Acid Mixtures

	Amounts given daily, gm.						
	Vaa	Vaf	Vag	Vah	Vuk	XVI	XVII
dl-Threonine	0.7	1.4	1.4	1.4	1.4	0.7	0.7
dl-Valine	1.5	3.0	3.0	3.0	1.8	1.5	1.5
l(−)-Leucine		2.2				1.1	1.1
dl-Leucine	2.2		3.0	3.0	2.0		
dl-Isoleucine	1.0	2.0	2.0	2.0	1.4	1.0	1.0
l(+)-Lysine·HCl·H₂O	1.1	2.2	2.2	2.2	1.6	1.1	1.1
dl-Tryptophane	0.3	0.3	0.3	0.6	0.24	0.3	0.3
dl-Phenylalanine	1.0	2.0	2.0	2.0	0.9	1.0	1.0
dl-Methionine	0.6	1.2	1.2	1.2	0.8	0.6	0.6
l(+)-Histidine·HCl·H₂O	0.5	1.0	1.0	1.0	0.5	0.5	0.5
l(+)-Arginine·HCl	0.5	1.0	1.0	1.0	1.0	0.5	0.5
Glycine	1.0	2.0	2.0	2.0	1.3	1.0	1.0
dl-Alanine						0.4	0.4
dl-Serine						0.2	0.2
dl-Aspartic acid						0.6	
dl-Norleucine						0.1	0.1
l(−)-Proline						1.5	1.5
l(−)-Hydroxyproline						0.1	0.1
l(−)-Cystine						0.05	0.05
l(−)-Tyrosine						0.1	0.1

EXPERIMENTAL OBSERVATIONS

In the reporting of these chronic plasmapheresis experiments the daily data are put into weekly averages or totals. Under the *diet* column are included the parenteral injections designated *s.c.* for subcutaneously and *i.v.* for intravenously; the remaining intake was given orally. Under *nitrogen balance* the intake item *R.B.C.* refers to the nitrogen of the *excess or deficit* of washed red blood cells in the plasmapheresis exchange procedure (7).

Tables 2 and 2-*a* demonstrate plasma protein production, nitrogen balance, and weight maintenance during a long period of amino acid administration.

The amino acids were the ten essentials plus glycine, the exact mixtures being given in Table 1. They were given subcutaneously, intravenously, and orally. During one week whole commercial casein substituted for amino acids and for two other periods an hemoglobin digest was fed.

Periods 1 to 3 on the basal diet only accomplished the depletion of the plasma protein reserves and the establishment of a circulating plasma protein level about 4 per cent. A weight loss of 10 per cent occurred, which was regained during subsequent amino acid periods 4 to 12. The nitrogen in the vitamin supplements of dried yeast and dried liver fraction does not appear to be an important protein supplement from previous experiments (1).

Periods 4 and 5 showed little production of plasma protein probably because the mixture Vaa was given in inadequate quantity.

In periods 6 and 7 small quantities of non-essential amino acids added to the mixture given the preceding 2 weeks were without benefit. In fact the *aspartic acid* of mixture XVI *induced vomiting* (8). There was vomiting on both occasions when it was present in the mixture (XVI) with injection rates of 10 and 7 mg. nitrogen/kilo/minute and no vomiting when it was removed (XVII) although nine of the subsequent twelve injections were at rates greater than 13 mg. nitrogen/kilo/minute and only one as slow as 7 mg. nitrogen/kilo/minute.

Periods 8 to 12 showed little improvement in plasma protein production but considerable gain in weight and increased nitrogen retention. It would appear that in this instance tissue protein had a higher priority for the incoming nitrogen.

In period 13 this tissue demand appeared satisfied and plasma protein accumulated and was removed from the circulation when casein was fed.

Periods 14 to 21 compare the influence of *changing the route of administration of amino acids*. Better utilization of orally administered amino acids appears definite from the lower urinary nitrogen and the higher plasma protein output. The periods of subcutaneous injection 20 and 21 show a little better efficiency than those of intravenous injection, 18 and 19. These differences may possibly be explained as differences in rates of inflow of amino acids. Half the injections by vein were done in 20 minutes or less (14.4 mg. nitrogen/kilo/minute) and all within 35 minutes. Subcutaneously the injections centered at 30 minutes (9.2 mg. nitrogen/kilo/minute), and would be further delayed in reaching the general circulation. It is of some interest that such rapid injections are as well utilized as they are.

Periods 22 and 23 tested a hemoglobin digest of low potency in plasma protein production. The negative nitrogen balance and the weight loss reveal the influence of the material more clearly than the amount of protein removed. Spoon-feeding was necessary and vomiting was frequent.

Periods 24 to 28 present an experiment in the *omission of histidine* in which

plasma protein production appears greater without histidine than with it. The quantities given of the amino acid mixture were two-thirds greater than those

TABLE 2

Plasma Protein Production from Amino Acids

Oral and Parenteral Administration Compared

Dog 42-1081

Period 7 days	Diet (amino acid mixtures given in Table 1)	Intake N × 6.25		Plasma protein removed total	Plasma protein level average	R.B.C. hemato-crit average	Weight
		Amino acids	Basal				
		gm.	*gm.*	*gm.*	*per cent*	*per cent*	*kg.*
	Initial				5.44	47.0	8.4
1	Low protein	0	9	15.0	5.00	50.3	8.4
2	Low protein	0	9	14.1	4.23	50.5	8.2
3	Low protein	0	22	5.6	3.99	47.9	7.6
4	Vaa, s.c.	59	22	4.1	3.87	47.0	7.6
5	Vaa, s.c.	59	22	7.6	3.92	45.3	7.7
6	XVI–XVII, i.v.	69	22	5.8	3.99	47.0	7.7
7	XVII, i.v.	67	22	2.6	4.08	47.3	7.7
8	Vag, s.c.	111	22	9.4	4.12	46.0	8.1
9	Vag, s.c.	111	22	12.4	3.94	47.1	8.4
10	Vag, s.c.	111	22	8.1	3.93	45.9	8.7
11	Vah, s.c.	112	22	8.8	3.90	44.2	8.7
12	Vah, s.c.	112	22	5.1	3.95	46.5	9.0
13	Casein	0	132	19.4	4.33	50.1	8.8
14	Vah, s.c.	112	22	13.9	4.03	50.1	9.1
15	Vah, s.c.	112	22	13.2	4.15	48.2	9.1
16	Vah, oral	112	22	15.2	4.14	50.7	9.0
17	Vah, oral	112	22	24.7	4.30	52.2	9.0
18	Vah, i.v.	112	22	14.9	4.19	49.3	8.9
19	Vah, i.v.	112	22	15.8	4.11	48.4	8.9
20	Vah, s.c.	112	22	17.9	4.12	50.0	9.3
21	Vah, s.c.	112	22	14.7	4.10	50.1	9.4
22	Hemoglobin digest, oral	90	17	11.4	3.93	49.2	9.1
23	Hemoglobin digest, oral	107	21	9.8	4.03	49.3	8.9
24	Vuk, oral	128	22	11.9	4.14	49.3	8.5
25	Vuk minus histidine, oral	104	19	14.5	4.15	49.3	8.3
26	Vuk minus histidine, s.c.	121	7	22.3	4.16	47.7	8.1
27	Vuk, s.c.	128	9	13.4	4.01	47.0	7.8
28	Vuk, s.c.	128	22	10.9	3.89	48.4	8.2

listed in Table 1. When fed in period 24 nitrogen retention was good. During the 1st week of feeding minus histidine *urinary nitrogen rose* slightly and during the 2nd week upon subcutaneous administration it rose sharply. Plasma protein production also rose and little weight loss occurred. In the following two periods the weight was partially regained but plasma protein production was poor. The Vuk mixture appears less effective than the Vah mixture,

although it is fair to call attention to the greater liver function impairment probably existing during the Vuk tests (see experimental history).

TABLE 2-a

Nitrogen Balance during Long Periods of Amino Acid Intake

Dog 42-1081

Period 7 days	Diet (amino acid mixtures given in Table 1)	Nitrogen						
		Intake			Output			Balance
		Amino acids	Basal	R.B.C.	Plasma	Feces	Urine	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	Low protein	0	1.4	0.3	2.5	—	11.5	−12.3
2	Low protein	0	1.4	−3.4	2.3	1.9	10.7	−16.9
3	Low protein	0	3.6	0.9	1.0	1.5	11.1	−9.1
4	Vaa, s.c.	9.5	3.6	−1.2	0.7	0.7	11.2	−0.7
5	Vaa, s.c.	9.5	3.6	0.4	1.2	1.0	9.6	+1.7
6	XVI–XVII, i.v.	11.0	3.6	−0.8	0.9	0.8	11.5	+0.6
7	XVII, i.v.	10.8	3.6	−1.8	0.4	1.8	10.9	−0.5
8	Vag, s.c.	17.7	3.6	3.7	1.5	1.3	16.5	+5.7
9	Vag, s.c.	17.7	3.6	−1.9	2.0	0.7	14.8	+1.9
10	Vag, s.c.	17.7	3.6	0.8	1.3	0.7	15.9	+4.2
11	Vah, s.c.	18.0	3.6	0.1	1.5	0.6	17.9	+1.7
12	Vah, s.c.	18.0	3.6	4.2	0.8	0.4	14.5	+10.1
13	Casein	0	21.2	−1.7	3.2	0.8	13.0	+2.5
14	Vah, s.c.	18.0	3.6	2.7	2.3	0.9	20.6	+0.5
15	Vah, s.c.	18.0	3.6	1.5	2.2	1.0	14.4	+5.5
16	Vah, oral	18.0	3.6	1.2	2.5	0.5	12.3	+7.5
17	Vah, oral	18.0	3.6	−1.4	4.0	1.0	12.1	+3.1
18	Vah, i.v.	18.0	3.6	−3.5	2.4	0.9	17.0	−2.2
19	Vah, i.v.	18.0	3.6	2.2	2.6	0.7	18.0	+2.5
20	Vah, s.c.	18.0	3.6	2.4	2.9	1.2	15.1	+4.8
21	Vah, s.c.	18.0	3.6	−2.2	2.4	1.3	16.2	−0.5
22	Hemoglobin digest, oral	14.5	2.8	−1.1	1.9	1.9	18.2	−5.8
23	Hemoglobin digest, oral	17.1	3.3	−2.1	1.6	1.8	18.1	−3.2
24	Vuk, oral	20.5	3.6	−0.3	2.0	1.6	13.1	+7.1
25	Vuk minus histidine, oral	16.5	3.1	−0.3	2.4	1.1	·14.1	+1.7
26	Vuk minus histidine, s.c.	19.3	1.2	2.9	3.6	—	22.6	−2.8
27	Vuk, s.c.	20.5	1.4	0.5	2.2	0.8	20.6	−1.2
28	Vuk, s.c.	20.5	3.6	0.4	1.8	1.7	18.3	+2.7
Totals.................		402.8	107.8	2.5	56.1	28.6	419.8	+8.6

Skin lesions and liver function impairment have occurred in previous experiments with similar synthetic diets. We have wondered how much of these abnormalitites to relate to the amino acids. From the observations of this report we believe little or none of the skin or liver change is produced by the amino acids.

Liver powder and yeast powder plus the vitamin emulsion described under Methods were the vitamin sources throughout the 28 weeks. A very few small skin lesions appeared near the end of the experiment but healed before the end. The same dietary regime was continued for another 4 weeks, now without plasmapheresis. At this time casein replaced the amino acids and the yeast and liver powders were omitted. Within 4 weeks skin lesions appeared. Similar lesions developed during the experiments of Tables 3 and 3-a when no liver powder and yeast powder were given. They did not clear when large amounts of casein were fed but promptly cleared on a mixed natural food diet (see also experimental history dog 42-893).

Experimental History—Tables 2 and 2-a.

Dog 42-1081. An adult female beagle hound was fed dextrose 105, corn oil 9, crisco 18, cod liver oil 3, yeast powder 3, liver powder 3, salt mixture (9) 4.5, and bone ash 4.5 plus vitamin emulsion (MH43-2231) 5 cc. during the entire experiment. When amino acids or casein was added the dextrose was reduced isocalorically.

Periods 1 and 2: Partial consumption of basal diet.

Periods 3 to 21: Complete voluntary consumption of basal diet.

Period 6: *Vomiting* of fluid and mucus occurred during injection of amino acid mixture XVI (pH adjusted to 6.5) on the first 2 days, consequently mixture XVII (pH 4.8) was given on the last 5 days and during period 7, without vomiting. The vomiting was probably a reaction to the dicarboxylic amino acid, *aspartic acid* (8).

Period 13: Commercial casein, 18 gm. per day, replaced the amino acids.

Period 15: An infestation of dog ticks was fairly well combatted by mechanical picking, washing, applying of the best available though very weak rotenone solution.

Period 17: The hair appeared slightly thinner than in the beginning.

Periods 22 and 23: Hemoglobin digest (Arlington Chemical Co. No. 1166), nitrogen 9.4 per cent, was spoon-fed against resistance and vomiting.

Period 24: Complete voluntary food consumption. At the end of this period bilirubin excretion test showed 29 per cent retention after 60 minutes. This is beyond the range of normal of 16 to 20 per cent. We are indebted to Dr. L. L. Miller for this and other such tests in this report.

Periods 25 to 27: When histidine was omitted the appetite lagged and the basal diet was partially spoon-fed, partially vomited. Near the end of period 27 appetite and voluntary consumption were renewed. During period 26 a few small, 3 to 4 mm., shallow ulcers appeared over the chest but these promptly healed in period 27 without local treatment.

Period 28: The dog was active, responsive, had a good coat of hair, and generally excellent condition at the close of this experiment.

After discontinuing plasmapheresis at the close of period 28 an amino acid intake and the basal diet were continued through period 32. There were now two small dry crusted areas on each leg but otherwise the skin and hair condition appeared good.

The liver and yeast powders were now omitted from the diet, emulsion MH43-2231, 5 cc., was retained, and vitamin-tested casein (S.M.A. Co.) 20 gm. now replaced the amino acids. Within 4 weeks the hair thinned considerably and a patchy reddening of the skin appeared, particularly over the pressure points and the distal third of the tail, now almost hairless. In the 36th week the diet was changed to hospital table scraps and the skin lesions promptly disappeared.

Tables 3 and 3-a (Dog 42-893) show continued plasma protein production from parenteral amino acid mixtures during an non-protein dietary deficiency with weight loss. The weight loss was accelerated by periods testing the effect of omitting the essential amino acids tryptophane, leucine, and isoleucine. The non-protein dietary deficiency was similar to that described in dog 42-1081 (Table 2). Skin lesions developed early in the course of this experiment since the vitamin intake was more restricted. Liver function impairment occurred. Neither skin lesions nor liver function were improved by casein feeding, beginning in period 25, but both were corrected when a mixed diet of natural foods was provided in period 40 (see experimental history dog 42-893).

Periods 1 to 3 of plasmapheresis during very low protein intake accomplished only partial depletion of the protein reserves. In periods 4 and 5 plasma protein production was unexpectedly high with the amino acid mixture Vaa. By comparison with the response to this mixture in Table 2 it appeared that some reserve protein supplemented this output. A slight decline in output and average plasma protein level occurred in period 6 but nitrogen balance and weight equilibrium are noted.

The *tryptophane deficiency* of period 7 provided a sharp drop in plasma protein output and circulating level. We question the recorded low urinary nitrogen output but know of no loss or error. Return of tryptophane to the intake of periods 8 and 9 did not return production of plasma protein to its orginal level but a definite rise occurred. There was weight loss and a slight negative balance.

In periods 10 and 11 the doubling mixture Vag of the amino acid intake (except for leucine and tryptophane) was followed (period 11) by a good rise in protein production. This was sustained and improved in periods 12 and 13 when the tryptophane was also doubled. Strong positive nitrogen balance was attained. Because of the production boost of period 11 it appears that the limiting factor of mixture Vaa is not tryptophane.

Periods 14 to 18 are of considerable interest. *Elimination of leucine and isoleucine* brought less severe decline in plasma protein production than was anticipated, but the rise in urinary nitrogen and the weight loss were considerable. *Return of isoleucine* to the intake (periods 17 and 18) brought return of abundant plasma protein production despite the still existing leucine deficiency. The continued weight loss and the even higher nitrogen loss may indicate raiding of body protein for the leucine needed in plasma protein regeneration.

Increased red blood cell destruction with hemoglobin splitting may have provided the leucine required for much of the plasma protein formed in periods 14 to 18. A large excess of washed red blood cells was returned during these periods (Table 3-a). A definite plasma icterus is recorded in the experimental history. We do not know whether this interesting reaction may be specific for

leucine deficiency during stimulus to plasma production or whether it was favored chiefly by the progress of the deficiency disease described in the experimental history below.

TABLE 3

Plasma Protein Production from Amino Acids during Vitamin Deficiency
Declines during Tryptophane, Leucine, and Isoleucine, or Leucine Omission

Dog 42-893

Period 7 days	Diet (amino acid mixtures given in Table 1)	Intake N × 6.25		Plasma protein removed total	Plasma protein level average	R.B.C. hemato-crit average	Weight
		Amino acids	Basal				
		gm.	gm.	gm.	per cent	per cent	kg.
	Initial				5.74	52.2	12.0
1	Low protein	0	30	5.3	5.08	51.3	11.2
2	Low protein	0	30	11.6	4.52	50.0	11.1
3	Low protein	0	30	19.6	4.09	47.7	10.8
4	Vaa, s.c.	59	30	20.2	4.20	48.9	10.8
5	Vaa, s.c.	59	30	23.5	4.08	49.1	10.7
6	Vaa, s.c.	59	0	19.4	3.86	48.7	10.7
7	Vaa, minus tryptophane, s.c.	57	0	7.2	3.64	49.2	10.6
8	Vaa, s.c.	59	0	12.2	4.00	47.5	10.4
9	Vaa, s.c.	59	0	8.6	3.95	47.2	10.2
10	Vaf–Vag, s.c.	111	0	9.6	4.10	44.7	10.3
11	Vag, s.c.	111	0	21.5	4.27	44.5	10.3
12	Vah, s.c.	112	0	20.8	4.32	47.9	10.4
13	Vah, s.c.	112	0	30.6	4.10	44.3	10.2
14	Vah minus leucine	89	0	19.4	4.15	40.8	9.7
15	and isoleucine, s.c.	89	0	14.6	4.01	45.7	9.5
16	(periods 14–16)	89	0	9.6	4.05	45.3	9.1
17	Vah minus	98	0	20.1	4.27	42.4	8.8
18	leucine, s.c.	98	0	24.2	4.22	43.5	8.3
19	Proteinless	0	0	1.4	4.46	40.6	7.5
20	Vah minus leucine, s.c.	98	0	0.7	4.77	34.3	7.3
21	Vah, s.c.	112	0	0.4	4.56	29.4	7.6
22	Vah, s.c.	112	0	0.4	5.07	28.7	7.6
23	Vah, s.c.	112	0	0.4	4.56	28.0	7.6
24	Vah, s.c.	112	0	0.9	4.86	26.3	7.6
25	Casein	0	122	0.5	5.04	29.6	7.5

The dietary deficiency rapidly developed in dog 42-893 after removal of the yeast and liver powders from the basal diet (period 6, Table 3). This appears most likely to have been a deficiency of essential metabolites other than amino acids. Choline 50 mg. daily appeared inadequate. Although 0.6 to 1.2 gm. of methionine may be equivalent to 180 to 375 mg. of choline, these quantities represent total methionine intake much of which may not have been available

for methyl donation. Liver function impairment might well be expected with a choline deficiency. The skin lesions may be evidence of deficiency of un-

TABLE 3-a

Nitrogen Balance during Amino Acid Omissions and Vitamin Deficiency

Dog 42-893

Period 7 days	Diet (amino acid mixtures given in Table 1)	Nitrogen						
		Intake			Output			Balance
		Amino acids	Basal	R.B.C.	Plasma	Feces	Urine	
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	Low protein	0	4.8	0.0	0.8	1.3	12.7	−10.0
2	Low protein	0	4.8	−2.5	1.9	1.2	11.7	−12.5
3	Low protein	0	4.8	−1.9	3.2	1.3	10.4	−12.0
4	Vaa, s.c.	9.5	4.8	3.6	3.2	0.4	9.5	+4.8
5	Vaa, s.c.	9.5	4.8	0.5	3.9	1.1	10.3	−0.5
6	Vaa, s.c.	9.5	0	2.8	3.2	0.5	7.6	+1.0
7	Vaa minus tryptophane, s.c.	9.2	0	0.8	1.2	0.5	5.1	+3.2
8	Vaa, s.c.	9.5	0	0.1	2.0	0.5	8.5	−1.4
9	Vaa, s.c.	9.5	0	−2.4	1.4	0.8	6.8	−1.9
10	Vaf–Vag, s.c.	17.6	0	3.5	1.6	1.0	10.5	+8.0
11	Vag, s.c.	17.7	0	1.9	3.5	1.7	11.4	+3.0
12	Vah, s.c.	18.0	0	1.0	3.4	0.9	12.5	+2.2
13	Vah, s.c.	18.0	0	2.1	5.0	1.1	12.9	+1.1
14	Vah minus leucine	14.2	0	2.6	3.2	0.5	17.4	−4.3
15	and isoleucine, s.c.	14.2	0	5.1	2.4	0.5	16.4	0.0
16	(periods 14–16)	14.2	0	1.3	1.6	0.5	16.2	−2.8
17	Vah minus	15.7	0	5.2	3.3	0.4	17.3	−0.1
18	leucine, s.c.	15.7	0	5.9	3.9	0.5	17.4	−0.2
19	Proteinless	0	0	−1.1	0.2	—	—	—
20	Vah minus leucine, s.c.	15.7	0	−0.4	0.1	*	13.0	+0.8
21	Vah, s.c.	18.0	0	−0.2	0.1	*	12.7	+3.6
22	Vah, s.c.	18.0	0	−0.2	0.1	*	10.8	+5.5
23	Vah, s.c.	18.0	0	−0.2	0.1	*	11.6	+4.7
24	Vah, s.c.	18.0	0	−0.4	0.1	*	9.0	+7.1
25	Casein	0	19.6	−0.2	0.1	*	5.8	+12.1
Totals............................		289.7	43.6	28.0	49.3	23.1	277.5	+11.4

* Estimated 1.4 gm.

saturated fatty acids or of still unknown factors. Certain unidentified factor(s) essential to the dog are known to be present in liver and yeast (2). We have earlier described skin lesions which healed promptly upon liver feeding (6).

The remaining periods, 19 to 25, of Tables 3 and 3-a, demonstrate little recovery from the deficiency state. Following cessation of plasmapheresis

(with red blood cell return) the hematocrit fell sharply, particularly during and immediately after period 20 of leucine omission. The long strenuous depletion of the 7 weeks of periods 14 to 20 was not corrected by the positive nitrogen balances of periods 21 to 25 and there was weight loss. The subsequent course described in the experimental history below shows that while weight and plasma protein level gradually rose during casein feeding the deficiency state was fundamentally corrected only when vitamins and other essential metabolites were supplied by a mixed diet. This deficiency may give

TABLE 4

Plasma Protein Production Decreased with Impaired Liver Function

Dog 41-187

Period 7 days	Diet (amino acid mixtures given in Table 1)	Intake N × 6.25		Plasma protein removed total	Plasma protein level average	R.B.C. hemato-crit average	Weight
		Amino acids	Basal				
		gm.	gm.	gm.	per cent	per cent	kg.
	Initial				5.16	39.5	12.2
1	Proteinless	0	0	13.9	4.76	43.4	11.8
2	Proteinless	0	0	9.0	3.90	46.5	11.5
3	Casein	0	122	11.1	4.00	45.0	11.5
4	Casein	0	122	16.9	4.05	44.7	11.7
5	Vuk, i.v.	77	0	9.6	3.94	45.1	11.6
6	Vuk, i.v.	77	0	6.1	3.96	48.4	11.5
7	Vuk, i.v.	96	0	9.3	3.97	47.5	11.4
8	Vuk, i.v.	96	0	5.4	3.88	42.4	11.4
9	Vuk, i.v.—casein digest, oral	117	0	9.4	4.04	43.6	11.7
10	Casein digest, oral	99	0	13.1	3.96	48.8	11.9
11	Amigen, oral	94	0	8.7	3.94	49.8	12.4
12	Amigen, oral	94	0	9.4	3.93	46.4	12.4
13	Amigen, oral	210	0	15.8	4.08	45.8	12.6
14	Amigen, oral	210	0	16.4	4.17	47.4	13.0

adequate explanation of the weight loss and low plasma protein production as evidenced by the very slow return of the plasma protein levels toward normal.

Experimental History—Tables 3 and 3-a.

Dog 42-893. An adult female mongrel received a synthetic diet of content in grams dextrose 140, corn oil 12, crisco 24, cod liver oil 4, yeast powder 4, liver powder 4, salt mixture (9) 6, and bone ash 6, plus vitamin emulsion (MH43-2231) 5 cc. during periods 1 to 5. Yeast and liver powders were omitted during periods 6 to 40, and inositol 50 mg. and *p*-aminobenzoic acid 35 mg. were added. Folic acid concentrate (Eli Lilly and Company) equivalent to 0.1 mg. folic acid was given daily during periods 22 to 40. The basal diet with vitamin supplements contained less than 0.1 gm. nitrogen per week after period 5 except when casein was added.

Periods 6 to 9: 80 per cent of the diet was consumed. In period 8 erythematous areas with hair loss appeared over two ankle joints and showed superficial ulceration a few days later.

Period 10: Mixture Vaf was given the 1st day and Vag for the remaining 6 days of this period.

Periods 10 to 20: Ulceration over pressure points became more widespread. Some ulcers healed then broke down again; some over the anterior thorax became as much as 3 cm. in diameter by period 20. The skin everywhere appeared dry and scaly and the hair became thin particularly over the head and tail. Tiny erythematous papules generously dotting the ventral surfaces are characteristic of this deficiency. The dog became generally listless,

TABLE 4-a

Positive Nitrogen Balance during Mediocre Plasma Protein Production

Dog 41-187

Period 7 days	Diet (amino acid mixture given in Table 1)	Nitrogen						
		Intake			Output			Balance
		Amino acids	Basal	R.B.C.	Plasma	Feces	Urine	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	Proteinless	0	0.2	10.0	2.3	0.3	10.2	−2.6
2	Proteinless	0	0.2	−1.2	1.5	0.7	6.6	−9.8
3	Casein	0	19.8	2.3	1.8	0.8	7.1	+12.4
4	Casein	0	19.8	1.8	2.8	0.9	12.4	+5.5
5	Vuk, i.v.	12.3	0.2	2.3	1.6	1.3	12.5	−0.6
6	Vuk, i.v.	12.3	0.2	3.5	1.0	0.9	9.9	+4.2
7	Vuk, i.v.	15.4	0.2	2.6	1.5	1.0	12.4	+3.3
8	Vuk, i.v.	15.4	0.2	−1.3	0.9	0.7	12.6	+0.1
9	Vuk, i.v.—casein digest, oral	18.7	0.2	6.0	1.6	1.1	8.6	+13.6
10	Casein digest, oral	15.8	0.2	1.4	2.2	1.8	11.1	+2.3
11	Amigen, oral	15.1	0.2	−1.9	1.4	1.7	9.6	+0.7
12	Amigen, oral	15.1	0.2	−1.0	2.0	1.2	11.5	−0.4
13	Amigen, oral	33.6	0.2	3.5	2.6	1.3	17.5	+15.9
14	Amigen, oral	33.6	0.2	2.5	3.8	1.5	18.0	+13.0
Totals..........................		187.3	42.0	30.5	27.0	15.2	160.0	+57.6

less active than at the start. Weight loss was considerable. The plasma had an icteric index of 3 to 4. Plasmapheresis was discontinued after period 18. Diet consumption improved to 100 per cent in period 18 and thereafter, whereas in periods 10 to 17 it had declined from 96 to 62 per cent.

Periods 21 to 24: There was no progress of skin lesions or of hair loss. All of the amino acid injections in this dog were given more rapidly than 3 mg. nitrogen/kilo/minute, most were greater than 6, and those of periods 21 to 24 were usually above 10.

Period 25: Commerical casein, 20 gm., was fed daily.

Periods 26 to 32: The regime was continued exactly as in period 25. During these 7 weeks the weight rose very slowly to 8.3 kilos and the plasma protein level to 6.00 per cent, the hematocrit to 37.7 per cent, with positive nitrogen balances of about 6 gm. per week. The dog gradually became more active and alert. The plasma icteric index dropped to the normal

zero level, but the bilirubin excretion test in period 29 showed 32 per cent retention after 60 minutes (normal 16 to 20). A new growth of hair began to appear and the ulcers improved slightly.

During periods 33 to 40 vitamin-tested casein (S.M.A. Co.) 40 gm. replaced the 20 gm. crude casein and while weight increased to 9.5 kilos by the 35th week the lesions of the skin remained unimproved. Specifically, the hair remained thin and short, particularly over ears, head, thighs, and tail; superficial ulcers over the head, ears, and chest were covered by crusts but a fresh ulcer 1 cm. in diameter appeared over the right thigh and the tail had weeping excoriated areas. The nails were brittle and split.

In the 40th week the weight was 9.3 kilos and the bilirubin excretion test still showed 33 per cent retention. The dog was placed on the kennel diet of hospital table scraps and 18 days later the bilirubin test dropped to 22 per cent retention—very close to normal. The skin lesions healed promptly.

Tables 4 and 4-a may suggest impaired liver function and related subnormal plasma protein regeneration. The dog had some evidence of liver function impairment at the start of this experiment, still present at the close (see experimental history below). The initial plasma protein level was low and the protein reserve rather small. Plasma production was fair in the 2nd week of casein feeding (period 4). It was poor with amino acids (periods 5 to 8) and very little better with casein digests fed during periods 9 to 14. Weight and nitrogen balance did increase with the digest feeding. Autopsy after period 14 showed only slight liver and kidney abnormalities. It is of interest that the daily choline (300 mg.) and inositol (50 mg.) given this dog for 14 weeks were not associated with any recognized improvement of liver function.

Experimental History—Tables 4 and 4-a.

Dog 41-187. An adult female mongrel, the subject of previous experiments (7, 5) was placed on a proteinless diet and plasmapheresis begun immediately at the close of the 15-week period on commercial casein already reported (7). During the casein 15-week period skin lesions similar to those described in dog 42-893 above were slowly healed. They were not well healed, however, and occasionally oozed and formed crusts. The hair coat was generally good but still showed a few bare areas. The bilirubin excretion test revealed a 42 per cent retention at the start of the experiment and the dog was languid. These observations suggest that this dog was suffering from some mild deficiency.

Periods 1 to 14: The basal diet contained in grams dextrose 147, corn oil 13, crisco 25, cod liver oil 5, salt mixture (9) 6, bone ash 6, vitamin emulsion (MH43-8111) 10 cc. Folic acid concentrate (equivalent to 0.1 gm. folic acid) was added daily after period 4.

Periods 3 and 4: Commerical casein 20 gm. per day was fed. A few small skin lesions remained.

Period 6: Vomiting occurred once during injection of Vuk at a rate of 5.1 mg. nitrogen/kilo/minute. Previous and subsequent injections produced no vomiting.

Period 8: Skin lesions remained healed for the rest of the experiment.

Period 9: Mixture Vuk was given the 1st day but casein digest (Eli Lilly and Company) was fed the remaining 6 days and all of period 10. All of the amino acid injections of periods 5 to 9 were given more rapidly than 4 mg. nitrogen/kilo/minute.

Periods 11 to 14: Amigen, an enzymatic digest of casein and pork pancreas, was fed. Two bilirubin excretion tests during period 11 each showed 38 per cent retention.

A fatal accident terminated the observations at the beginning of period 15. Autopsy showed normal organs in gross. Histological sections showed the liver to contain much hemosiderin in Kupfer cells and in clusters of monouclear phagocytes. Some liver cells showed large fat droplets but the majority of the liver cells were histologically normal. Fatty degeneration of straight tubules of the kidney cortex in some areas was marked. Other organs and tissues were negative.

DISCUSSION

Reference to the basic work of Professor Rose is inevitable in any discussion of amino acids in nutrition. For the growth of rats he has demonstrated the requirement for exogenous threonine, valine, leucine, isoleucine, lysine, tryptophane, phenylalanine, methionine, histidine, and arginine (10). For nitrogen balance in the dog he has found arginine unnecessary (11). With these leads we have been testing the requirement for plasma protein production in the dog. On theoretical grounds we did not expect the exogenous requirements to be less extensive than those for nitrogen balance, for homologous plasma may be given by vein in the absence of other protein nitrogen to produce nitrogen balance (3, 1). That they might be more extensive appeared entirely possible and that the quantity of individual amino acids needed for the abundant production of plasma protein might vary considerably from the basic demands of maintenance appeared very likely. We have found that abundant production of plasma protein occurs with administration of the ten amino acids essential for rat growth (5, 7). The evidence is suggestive that arginine is required for much plasma protein formation (5). Addition of amino acids other than these ten has not been clearly shown to speed up production (Table 2 above, and reference 1).

Although we have not expected fewer than the ten amino acids mentioned to be adequate for plasma formation, we have been interested in testing the effect of the withdrawal of individual amino acids from the mixture. It is admitted that interpretation of the specific influence of such withdrawal upon new plasma protein synthesis is difficult. When an essential for total body protein maintenance is lacking from the diet internal shifts of protein occur which may at least temporarily obscure certain of the effects. We have been interested in studying these shifts of protein in response to emergency demands. When *threonine* or *valine* was omitted the decline in plasma protein production was sharp within the 1st week and the urinary nitrogen was high (5). But there was little weight change.

When *leucine* and *isoleucine* were omitted (Tables 3 and 3-a above) the decline in plasma protein production was slow, running into the 3rd week but urinary nitrogen promptly exceeded the nitrogen intake and weight loss was sharp. Return of isoleucine to the intake brought forth excellent protein regeneration but high urinary nitrogen and weight loss continued during the leucine deficiency.

Omission of *lysine, histidine,* and *arginine* as a group promptly depressed plasma protein production, but nitrogen balance was positive despite some increase in urinary output (5).

Tryptophane omission early reduced plasma protein formation (Tables 3 and 3-*a* above). The low urinary nitrogen was unexpected and needs confirmation.

When *methionine* was omitted and cystine given, plasma protein production was excellent, but the urinary nitrogen jumped up and weight loss occurred (5).

When the tables are examined carefully during periods of an essential amino acid deficiency it will be noted that the output of plasma protein may be sustained for 1, 2, or even 3 weeks and we must assume that the body draws on some source supply of the missing amino acid without which prolonged production of new plasma protein is impossible. It appears probable that certain amino acids may be withdrawn from appropriate tissue protein without its destruction—further that amino acids resulting from normal body tissue wear and tear or incidentally accelerated catabolism may be recaptured. Whether protein may be actually destroyed to supply the deficient amino acids is an open question.

After this deficiency period when the missing amino acid is returned to the mixture or even when a complete protein is fed, we may note some *delay* in the output of new plasma protein—sometimes as much as 2 or 3 weeks. One may say that this delay means that in this emergency the tissue cells have a definite priority over plasma protein accumulation until the urgent deficiency is made up. One may choose to believe that the deficiency has actually disturbed the normal physiology of liver cells, skin epithelium, and other tissues. Repair is needed before normal plasma protein production goes forward and repair is delayed in the face of hypoproteinemia.

Liver function belongs in this discussion. It must be admitted that liver functional tests may be at times very misleading. The bilirubin excretion test is abnormal in some of the dogs described above but one cannot claim that this is a measure of the capacity of the liver to produce new plasma protein. Taken together with other evidence we may suspect that at times in these deficiency states the liver function is disturbed and may be the cause of the low plasma protein production.

In support of this suggestion we may mention observations in this laboratory (4, 12) on the Eck fistula to show definite incapacity of the Eck fistula dog to produce plasma protein and hemoglobin. There are only slight structural abnormalities noted in the Eck fistula liver but there can be little doubt that at times its function is gravely impaired. This may mean 25 per cent of normal capacity to produce hemoglobin in anemia and even 10 per cent of normal capacity to produce new plasma protein during periods of hypoproteinemia.

SUMMARY

When blood plasma proteins are depleted by bleeding with return of red cells suspended in saline (plasmapheresis) it is possible to bring dogs to a steady state of hypoproteinemia and a constant level of plasma protein production if the diet nitrogen intake is controlled and limited. Such dogs are outwardly normal but have a lowered resistance to infection and intoxication and probably to vitamin deficiency.

When the diet nitrogen is provided by certain mixtures of the ten growth essential amino acids plus glycine, given intravenously at a rapid rate, plasma protein production is good. The same mixture absorbed subcutaneously at a slower rate may be slightly better utilized. Fed orally the same mixture is better utilized and associated with a lower urinary nitrogen excretion.

An ample amino acid mixture for the daily intake of a 10 kilo dog may contain in grams dl-threonine 1.4, dl-valine 3, dl-leucine 3, dl-isoleucine 2, l(+)-lysine·HCl·H$_2$O 2.2, dl-tryptophane 0.3, dl-phenylalanine 2, dl-methionine 1.2, l(+)-histidine·HCl·H$_2$O 1, l(+)-arginine·HCl 1, and glycine 2. Half this quantity is inadequate and not improved by addition of a mixture of alanine, serine, norleucine, proline, hydroxyproline, and tyrosine totalling 1.4 gm.

Aspartic acid appears to induce vomiting when added to a mixture of amino acids. The same response has been reported for glutamic acid (8).

Omission from the intake of leucine or of leucine and isoleucine results in negative nitrogen balance and rapid weight loss but plasma protein production may be temporarily maintained. It is possible that leucine may be captured from red blood cell destruction.

Tryptophane deficiency causes an abrupt decline in plasma protein production. No decline occurred during 2 weeks of histidine deficiency but the urinary nitrogen increased to negative balance.

Plasma protein production may be impaired during conditions of dietary deficiency not related to the protein or amino acid intake. Skin lesions and liver function impairment are described. Unidentified factors present in liver and yeast appear to be involved.

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