TEN AMINO ACIDS ESSENTIAL FOR PLASMA PROTEIN PRODUCTION EFFECTIVE ORALLY OR INTRAVENOUSLY*‡

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The purpose of the present paper is to present experiments showing that the aggregation of ten amino acids adequate for growth in rats (Rose) when tested in standardized plasma depleted dogs produces new plasma proteins in abundance. In fact, this amino acid growth complex compares favorably with standard food proteins fed by mouth. The ten amino acids which produce abundant new plasma protein in these experiments are threonine, valine, leucine, isoleucine, lysine, tryptophane, phenylalanine, methionine, histidine, and arginine. These ten amino acids are as effective in plasma protein production when given by vein as by mouth, and in many periods of 1 to 6 weeks as the sole source of protein derivatives maintained health and nitrogen equilibrium (Tables 1 and 2). Given by vein this amino acid complex is better tolerated than any protein digest so far tested.

When we withdraw one or more amino acids from this growth complex, we may say that in the combinations tested there is a less favorable response. Either the production of plasma protein may falter or fail or the nitrogen balance becomes strongly negative with impaired appetite and a disturbed clinical state. It is possible that one or more of these ten amino acids may be dropped out without impairing the production of new plasma protein under these experimental conditions. While arginine might be omitted for short periods (Table 1) without reducing plasma protein production this withdrawal would be contraindicated in man as leading to testicular impairment (7). Omission of the trio of histidine, lysine, and arginine (Table 4) dropped plasma protein production toward zero, but nitrogen balance was maintained as has been reported for 8-day periods in adult rats (3). The most effective combination of specific amounts of each amino acid is not yet determined. Supplementing these ten amino acids by other amino acids (*e.g.* prolin) may give a more potent mixture for plasma protein production, cell repair, and nitrogen retention. All of these questions can be answered by suitable experiments, some of which are in progress.

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Methods

The principles and the details of the procedures used in these experiments have been previously described or referred to (10). Aseptic methods were employed in the plasmapheresis procedure and in the preparation and injection of the amino acid solutions.

Crystalline amino acids were given in the mixtures listed in Table 3. Sodium bicarbonate sufficient to neutralize the hydrochlorides and the glutamic acid was added to each mixture except those given intravenously. The hemoglobin digest, lot No. KB81341, was a papain digest of bovine hemoglobin, 13.1 per cent nitrogen. The casein digest was from the same lot prepared by papain digestion as that used in previous experiments (12) and contained 12.5 per cent nitrogen. The thymus, labeled "veal sweetbreads," a commercial canned product thawed from the frozen state and fed uncooked, had a nitrogen content of 2.71 per cent. The fresh beef pancreas contained 3.20 per cent nitrogen.

To supply the caloric, salt, and vitamin requirements three basal diets were employed. The items of *basal diet A* in grams were sucrose 85, corn starch 20, corn oil 15, crisco 15, cod liver oil 5, bone ash 10, salt mixture 5, yeast powder 2, liver powder 2, thiamin chloride 0.005, nicotinic acid 0.05, choline chloride 0.4. The liver and yeast powders and the salt mixture have been previously defined (12). The items of *basal diet B* were sucrose 85, corn starch 20, corn oil 20, crisco 15, bone ash 10, salt mixture 5, and *one daily quota of vitamins*, put up in four separate capsules to contain vitamin A 5,000 U.S.P. units, thiamin chloride 3 mg., riboflavin 2 mg., pyridoxine hydrochloride 1 mg., calcium pantothenate 1 mg., nicotinamide 20 mg., ascorbic acid 50 mg., vitamin D 500 U.S.P. units; mixed natural tocopherols 50 mg.; choline chloride 100 mg., 2-methyl 1,4 naphthoquinone 1 mg.; rice polish concentrate (free of vitamins B_1, B_2, and B_6) 500 mg. The *basal cake* was a mixture in parts per 100 of corn starch 38.6, sucrose 19.3, dextrin 6.4, crisco 12.9, corn oil 2.4, baking powder (alum type) 1.6, salt mixture (21) 1.2, bone ash 4.0, kaolin 4.0, water 9.6. This mixture was baked into a dry cake and allowing for moisture loss was calculated to contain 4.9 calories per gm. It was always fed with one daily quota of vitamins as listed above and was often voluntarily consumed when a similar mixture offered as a mush was refused. The vitamin capsules were fed separately if necessary in order to insure their intake.

EXPERIMENTAL OBSERVATIONS

The data recorded in the accompanying tables were obtained by averaging or totaling daily determinations into weekly periods. In the column for protein intake the figures represent protein nitrogen, amino acid nitrogen, or digest nitrogen intake multiplied by 6.25. In Tables 1 and 1-*a*, 2 and 2-*a*, the 30 weekly periods represent chronologically consecutive observations in dog 41-187. In Tables 4, 4-*a*, and 4-*b* are presented two segments of a 64-week continuous experiment in dog 40-122, the other portions of which are reported in a separate paper (8).

Summary of Tables 1 and 1-a.—Periods 1 to 4 show the initial plasma protein level (5.97 gm. per cent) and its depletion to the standard hypoproteinemia

level (4.00 gm. per cent). The liver diet shows an average plasma protein production of 17 gm. per 7 days, a ratio of about 4 gm. of liver protein intake to 1 gm. plasma protein output. During this period about 45 gm. of plasma protein is removed over and above that attributable to the liver protein fed (33 gm. during fasting plus 12 gm. above the liver output). This is termed the *reserve* store which is derived in part from the change in plasma levels (10 gm.) and in large part from organs and tissues. This *reserve* store of plasma protein building material should be regarded as actively metabolizing protein and not an inert mass of stored plasma protein (10, 16).

Periods 5 to 8 show the effect of *arginine* as a part of the *amino acid growth complex*. When arginine is left out of the amino acid mixture (periods 5 and 6) there is a slow fall in plasma protein output, and when arginine is replaced (periods 7 and 8) there is a slow return to a normal output. In all 4 periods, however, there is more nitrogen intake than urinary output—a positive nitrogen balance and no weight loss. *Arginine* when left out of the amino acid growth mixture causes least disturbance of any amino acid so far tested. For a time the body can do pretty well without arginine (15, 7).

Protein-free periods (9, 13 and 19) usually in the first 2 or 3 days show some "carry over" from the preceding diet period. When this "carry over" is exhausted (period 9—the first 2 days), the capacity of the body to form new plasma protein is obviously zero or, at the most, a gram or two.

Periods 10 and 22 show how effectively *cystine* can pinch-hit for methionine when replacing it in the amino acid growth complex. Nitrogen balance is not maintained and this cystine effect appears to be at the expense of body protein. There is a definite weight loss, too.

The *hemoglobin digest* fed in period 12 with *added cystine* was much better utilized for plasma protein production than any hemoglobin material previously tested (11, 9). That this effect may be due largely to the cystine in conjunction with body protein rather than the hemoglobin digest must be considered in view of the reaction in period 20. This digest was poorly tolerated by the dog and urinary nitrogen was high.

Periods 14 to 16 are resting periods on a high protein intake. The plasma protein level returns to normal and there is a gain in weight.

In periods 17 and 18 the removal of plasma protein surplus and reserve stores again established hypoproteinemia.

Periods 20 and 21 show the *cystine effect* after the ordinary depletion of the reserve stores (periods 17 to 19). The plasma protein output is considerable in period 20, but falls to zero in period 21. When the amino acid mixture (VI) with *cystine replacing methionine* is fed in period 22, we observe a considerable output of plasma protein but there is a loss of weight. Again the *cystine* influences internal body metabolism and for a time a surplus of plasma protein is formed.

Summary of Tables 2 and 2-a.—These tables continue without interruption the observations of Table 1 and are of particular interest because of the 6 weeks continuous intake of amino acids to replace all protein in the diet.

TABLE 1

Plasma Protein Production with Amino Acid Mixtures

Cystine Alone Extracts Considerable Plasma Protein

Dog 41-187.

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein removed Total for 7 days	Blood plasma Average concentration		R.B.C. hematocrit, average	Weight
				Total protein	A/G ratio		
		gm.	*gm.*	*per cent*		*per cent*	*kg.*
	Kennel			5.97	1.03	44.9	11.2
1	Fasting	0	33.0	5.60	0.85	45.8	9.9
2	Liver	85	27.9	4.46	0.70	45.0	10.4
3	Liver	70	16.0	4.08	0.71	45.6	10.2
4	Liver	70	18.8	4.04	0.90	48.8	10.3
5	Liver, 3 days; amino acids IV*a*, 4 days	93	17.4	3.99	1.01	50.7	—
6	Amino acids IV*a*	102	13.7	4.05	1.17	48.6	9.5
7	Amino acids V	116	7.3	4.06	1.13	48.7	9.8
8	Amino acids V	116	13.0	4.17	1.25	49.7	10.4
9*a*	Protein-free, 2 days	0	4.1	3.91*			
9*b*	Protein-free, 7 days	0	1.0	3.80*	0.93	49.7	10.3
10*a*	Amino acids VI, 3 days	41	0.6	4.32*	1.04		9.9
10*b*	Amino acids VI, 7 days	79	21.6	4.64*	1.20	45.0	9.1
11	Protein-free, 4 days	0	8.9	4.21*	1.28	45.5	—
12	Hemoglobin digest + cystine	80	16.2	4.24	0.89	48.0	9.5
13	Protein-free, 5 days	0	9.1	4.00	0.83	46.5	9.1
14	Liver-salmon-beef	519	14.9	4.79	0.97	45.0	—
15	Liver-salmon-beef	519	0.4	5.57	1.03	45.0	10.7
16	Liver-salmon-salmon bread	357	0.3	5.65	1.30	47.0	11.0
17	Liver	70	35.4	5.15	1.12	48.5	10.8
18	Liver	70	22.2	3.99	0.78	49.8	10.2
19	Protein-free	0	3.7	3.99	0.80	48.6	9.9
20	Protein-free + cystine	0	14.1	4.15	0.83	47.0	9.5
21	Protein-free + cystine	0	1.8	3.76	0.90	45.1	9.3
22	Amino acids VI	116	18.3	4.39	0.74	44.9	9.0

* Level at end of period.

Period 23 shows a considerable intake of *thymus* and a modest output of plasma protein. The thymus protein is about one-half as efficiently used as was the liver (Table 1)—about 8 gm. of thymus protein being required to

TABLE 1-*a*

Nitrogen Balance with Amino Acid Mixtures IV a and V

Minimum Nitrogen Catabolism Not Influenced by Amino Acids

Dog 41-187.

Period 7 days	Diet	Nitrogen balance							
		Intake		Output					Intake minus output
		In diet	In excess R.B.C. injected	In plasma	In feces	In urine			
						Total	Urea + NH_3	Undetermined	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*
	Kennel								
1	Fasting	0.0	1.3	5.4	0.0	17.2	92.6	1.30	−21.3
2	Liver	13.6	0.4	4.6	—	18.7	79.6	3.8	−11.6*
3	Liver	11.2	2.3	2.6	—	9.1	63.4	3.3	−0.5*
4	Liver	11.2	3.0	3.1	2.3	8.3	71.1	2.4	+0.5
5	Liver, 3 days, amino acids IV*a*, 4 days	14.8	−0.1	2.9	2.0	10.7	71.2	3.1	−0.9
6	Amino acids IV*a*	16.3	0.0	2.3	2.2	12.4	68.5	3.9	−0.6
7	Amino acids V	18.5	−2.6	1.2	2.7	14.7	72.5	4.1	−2.7
8	Amino acids V	18.5	−0.1	2.1	2.2	11.2	68.1	3.6	+2.9
9*a*	Protein-free, 2 days	0.0	0.6	0.7	‡	3.2	64.0	1.2	‡
9*b*	Protein-free, 7 days	0.0	−1.3	0.2	2.9	5.5	56.8	2.4	−13.2
10*a*	Amino acids VI, 3 days	6.6	−0.7	0.1	‡	5.8	67.5	1.9	‡
10*b*	Amino acids VI, 7 days	12.6	−0.2	3.5	2.8	14.8	64.7	5.2	−8.7
11	Protein-free, 4 days	0.0	1.0	1.4	‡	5.0	63.4	1.8	−5.4
12	Hemoglobin digest + cystine	13.4	0.9	2.7	2.3	13.2	72.6	3.6	−3.9
13	Protein-free, 5 days	0.0	1.9	1.5	1.6	8.0	69.0	1.0	−9.2
14	Liver-salmon-beef	83.1	2.9	2.4	—	—	—	—	—
15	Liver-salmon-beef	83.1	−0.3	0.1	—	—	—	—	—
16	Liver-salmon-salmon bread	58.8	−0.3	0.1	—	—	—	—	—
17	Liver	11.2	3.1	5.8	5.3	12.6	83.8	2.0	−9.4
18	Liver	11.2	−1.5	3.6	3.5	15.3	78.8	3.2	−12.7
19	Protein-free	0.1	−1.7	0.6	4.3	10.1	76.5	2.8	−16.6
20	Protein-free + cystine	1.1	−0.3	2.3	3.4	5.0	58.3	2.1	−9.9
21	Protein-free + cystine	1.1	−2.0	0.3	3.1	4.5	47.9	2.3	−8.8
22	Amino acids VI	18.8	3.2	3.0	2.5	18.7	65.7	6.4	−2.2
	Totals (except periods 14–16)	180.2	7.2	49.9	47.7	224.0			−134.2

* Assumed fecal nitrogen = 2.3 gm.

‡ Included in next period.

produce 1 gm. of plasma protein. Thymus is rich in nucleoprotein and this substance evidently has no conspicuous potency for plasma protein production.

Period 24 eliminates the "carry over" from the thymus period and indicates that *methionine* does not show the "cystine effect" but this length period is not a convincing test of this point.

Periods 25 and 26 show well the potency of the *amino acid mixture* "adequate for growth." The dog gains weight, is in positive nitrogen balance, and produces 15 gm. of new plasma protein each week.

TABLE 2

Amino Acids Given for 6 Consecutive Weeks

Potent by Vein As by Mouth

Threonine Essential

Dog 41-187.

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein removed Total for 7 days	Blood plasma Average concentration		R.B.C. hematocrit, average	Weight
				Total protein	A/G ratio		
		gm.	*gm.*	*per cent*		*per cent*	*kg.*
23	Thymus	178	22.5	4.31	0.85	46.6	9.0
24*a*	Protein-free, 4 days	0	4.1	3.52			
24*b*	Protein-free + methionine, 3 days	0	1.0	3.69	0.55	46.4	8.9
25	Amino acids V*a*	114	15.3	4.27	0.69	47.6	9.2
26	Amino acids V*a*	114	15.3	4.10	0.74	47.6	9.3
27	Amino acids V*b* minus glycine	78	17.6	4.01	0.77	46.6	9.0
28	Amino acids V*b* minus threonine	113	8.4	3.90	0.78	47.3	9.2
29	Amino acids V*b* by vein	104	16.3	4.27	0.72	49.2	9.0
30	Amino acids V*b*—VII by vein	114	16.1	4.01	0.75	47.7	8.4

Period 27 shows essentially the same amino acid growth mixture *minus glycine* to be very potent in plasma protein production. The nitrogen intake is quite low and there is a little body weight loss but a positive nitrogen balance continues.

Period 28 shows that *threonine* is truly an essential member of the amino acid plasma protein production complex. During this period there is a great rise in urinary nitrogen—a negative nitrogen balance, and a rapid fall toward zero of the plasma protein output. There was a loss of appetite yet there was no weight loss.

Periods 29 and 30 show the result of the growth mixture of *amino acids*

given by vein. There was not the slightest reaction to these considerable intravenous injections (period 29) even when given rapidly. The output of plasma proteins is impressive and the urinary nitrogen did not exceed the nitrogen in the injected amino acids. During period 30 the increase in the essential amino acids at the expense of glycine (mixture VII) was not followed by any better utilization. In fact, as noted in the Experimental History, some reaction occurred to the injection, not avoided by slowing the rate of injection from 40 to 90 minutes, but entirely relieved following reduction of the trypto-

TABLE 2-*a*

Excellent Nitrogen Retention with Oral Amino Acids

Dog 41-187.

Period 7 days	Diet	Nitrogen balance							
		Intake		Output					Intake minus output
		In diet	In excess R.B.C. injected	In plasma	In feces	In urine			
						Total	Urea + NH_3	Undetermined	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*
23	Thymus	28.5	1.9	3.7	5.8	17.7	77.8	3.9	+3.2
24	Protein-free, 4 days; protein-free + methionine, 3 days	0.5	−1.1	0.8	3.7	6.4	61.9	2.4	−11.5
25	Amino acids V*a*	18.6	3.1	2.5	3.7	7.7	47.8	4.0	+6.8
26	Amino acids V*a*	18.6	0.8	2.5	3.0	9.0	52.8	4.2	+4.9
27	Amino acids V*b* minus glycine	12.7	5.2	2.9	3.1	7.9	34.3	5.2	+4.0
28	Amino acids V*b* minus threonine	18.4	0.6	1.4	3.2	17.4	67.0	5.7	−3.0
29	Amino acids V*b* by vein	17.0	1.0	2.7	3.6	17.1	55.2	7.7	−5.4
30	Amino acids V*b*—VII by vein	18.6	1.2	2.7	1.5	15.5	60.7	6.1	+0.1
Totals (periods 23–30)		132.9	12.7	19.2	27.6	98.7			+0.1

phane to its former level. Whether the high tryptophane intake is the significant factor in the reaction to the injection mixture will be further investigated.

During the 6 consecutive weeks of amino acid intake, periods 25 to 30, *weight and nitrogen equilibrium* were well maintained. The weight loss in period 30 was partly if not entirely due to a low caloric intake (see Experimental History, dog 41-187).

More globulin by weight than albumin was produced in most periods. The albumin:globulin ratio rose above 1 during the first amino acid feeding days, periods 5 to 8, and again in period 10, but failed to rise significantly during

the later days of periods 25 to 30. Again in dog 40-122, Table 4, certain amino acid feeding was associated with a rise in albumin production, periods 26 to 28 and period 59, but was not sustained in period 60 and at no time equal to globulin production. It is proper to note, however, that heavy protein feeding in comparable periods, Tables 1, 2, and 4, is no more successful than the potent amino acid mixtures in albumin production.

TABLE 3

Amino Acid Mixtures

	Amounts given daily									
	I	II	III	IV	IV*a*	V	V *a*	V *b*	VI	VII
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1. *dl*-Threonine	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	2.5
2. *dl*-Valine	1.0	1.0	1.0	1.0	1.0	1.0	1.5	1.5	1.5	2.5
3. *l*(−)-Leucine	3.0	3.0	3.0	3.0	3.0	3.0	1.5			
4. *dl*-Leucine								3.0	3.0	3.0
5. *dl*-Isoleucine	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	2.0
6. *l*(+)-Lysine	0.1*	0.1*	1.5*	1.8‡						
7. *dl*-Lysine*					1.5	1.5	3.0	3.0	3.0	3.0
8. *l*(−)-Tryptophane	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.8
9. *dl*-β-Phenylalanine	0.2	0.2	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0
10. *dl*-Methionine	0.4	0.4	0.4	1.0	1.0	1.0	1.25	1.25		2.5
11. *l*(+)-Histidine*			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
12. *l*(+)-Arginine*			0.5			0.5	0.5	0.5	0.5	1.5
13. *l*(−)-Cystine	0.7	0.7	0.7						1.0	
14. *l*(−)-Tyrosine	1.0	1.0	1.0							
15. Glycine	2.7	3.8	1.1	6.4	6.4	6.4	5.4	5.4	5.4	
16. *dl*-Alanine	1.3	2.0	2.0							
17. *l*(−)-Aspartic acid	3.0									
18. *l*(+)-Glutamic acid	5.2	5.2	5.2							

* As monohydrochloride.

‡ As dihydrochloride.

Experimental History—Tables 1 and 1-*a*, 2 and 2-*a*. *Dog 41-187*, an adult female mongrel hound was fed as listed in the tables. The liver diet of period 2 consisted daily of raw pork liver, 50 gm., plus basal diet A. During periods 3 through 13, to basal diet B the liver and the amino acid mixtures were added as indicated in the tables.

Half of 1 day's diet was vomited in period 6 and part of it had to be spoon fed in the next period, all of it in the following period. The basal diet, however, was eaten voluntarily in period 9. When amino acids VI was added in period 10, spoon feeding was again necessary and some vomiting dropped the net intake to 68 per cent. Choline chloride was increased to 0.4 gm. daily for period 10.

Hemoglobin digest, 20 gm., and *l*-cystine, 0.6 gm., were added to the basal diet in period 12. Spoon feeding was necessary and vomiting occurred. Clean, punched-

out ulcers of the skin over the bony prominences began to appear and the dog was less active clinically. In period 14 after adding pork liver 50 gm., canned salmon 100 gm., and lean beef muscle 200 gm. to basal diet B, diarrhea commenced, became bloody in the gross, then cleared spontaneously in period 15, as did the skin ulcers. In the 16th week, 200 gm. salmon bread (21) replaced the beef muscle and the depleted state was further corrected.

The basal cake was started in period 17, 155 gm. per day, entirely consumed through period 20, 85 per cent consumption in period 21. In these latter two periods *l*(−)-cystine, 1.0 gm., was added daily; choline chloride was increased to 0.4 gm. daily and continued at this level through period 30.

Amino acids VI mixed into 30 gm. of a corn oil-crisco, soft paste was retained 100 per cent when spoon fed in period 22 but since no basal cake was eaten voluntarily the first 3 days, 100 gm. per day was spoon fed during the remaining 4 days.

Thymus 150 gm., uncooked, was relished daily in period 23 when mixed with the basal cake. During the last 3 days of period 24 *dl*-methionine 1.25 gm. daily was added.

The amino acid mixtures of periods 25, 26, 27, and 28 were spoon fed mixed with corn oil and crisco, and the basal cake was eaten largely voluntarily.

The mixture of amino acids V*b* in 400 cc. solution was injected first during 90 minutes time, gradually reduced on successive days to 30 minutes time, always without the slightest evidence of clinical disturbance. When amino acids VII was injected in 40 minutes on the 3rd day of period 30, the dog vomited and defecated, and vomited once on each of the succeeding 2 days. No vomiting and no other reaction occurred, however, when the tryptophane content of the mixture was reduced from 0.8 gm. to 0.4 gm. on each of the final 2 days. On the final day *l*(−)-leucine, 1.5 gm., replaced *dl*-leucine, 3.0 gm. Basal cake, 155 gm., was given daily during periods 29 and 30 with some spoon feeding in period 29. Lacking spoon feeding, consumption dropped to 45 per cent in period 30.

At the close of the experiment, the dog was in good clinical condition although not as sleek as 2 weeks later following liver feeding. A scaliness and redness of the skin of the abdomen and the chest gradually appeared during the 26th period. Erythematous areas appeared over the joint surfaces, and one developed an ulcerated center. The tip of the tail reddened and crusted. Hair generally became sparse. Double quantities of the vitamin mixture were given in periods 29 and 30 without apparent improvement but after liver feeding the skin became normal again. A vitamin limitation is suggested.

Summary of Tables 4, 4-a, and 4-b.—Dog 40-122 was in good condition during a 64 week continuous plasma depletion. The portions of this experiment concerned with protein digests are reported elsewhere (8). The periods dealing with *amino acids* are given in these tables.

Periods 23 to 25 are given in detail in Table 4-*b* and show conclusively that the growth mixture *minus* lysine (0.1 gm.), histidine, and arginine is inadequate for plasma protein production. When the *casein digest* replaces the amino acid mixture we observe a slow uptake in plasma protein production (period 24). A similar amino acid mixture (period 25) again shows a prompt

decline in the output of plasma protein. However, there is nitrogen equilibrium. From other experiments (Table 1) we assume that the amino acids tyrosine, glycine, alanine, aspartic and glutamic acids are unessential in mixtures I and II.

Periods 26 and 27 show by contrast the response to the *growth complex of*

TABLE 4

Plasma Protein Production Obtained from Amino Acid Mixtures Poor without Lysine, Histidine, and Arginine Valine Appears Essential

Dog 40-122.

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein removed Total for 7 days	Blood plasma average concentration		R.B.C. hematocrit average	Weight
				Total protein	A/G ratio		
		gm.	*gm.*	*per cent*		*per cent*	*kg.*
23	Amino acids I	104	14.0	3.91	0.37	41.7	10.6
24	Casein digest by mouth	110	8.7	3.98	0.44	43.7	10.3
25	Amino acids II	112	6.4	3.83	0.46	41.9	10.4
26	Amino acids III	113	11.2	4.04	0.54	45.8	10.4
27	Amino acids III	113	16.0	4.11	0.70	45.2	10.9
28	Amino acids IV	48	12.8	3.95	0.86	44.2	10.3
29	Low protein	15	1.6	3.60	0.73	44.7	10.4
30	Amino acids IV	73	1.6	3.76	0.67	43.8	10.1
31	Low protein	15	1.7	3.85	0.69	41.7	9.7
32	Casein digest by mouth	109	3.9	4.07	0.53	38.4	10.2
33	Casein digest by mouth	109	21.1	4.17	0.47	37.9	10.3
34	Fasting	0	2.2	3.99	0.40	37.3	9.0
35	Amino acids IV Liver	46 30	11.9	4.03	0.40	38.3	9.2
56	Protein-free, 2 days	0	1.1	3.90	0.71	41.4	8.6
	Pancreas, 5 days	150	11.8	4.69			
57	Pancreas	210	28.5	4.33	0.85	40.5	9.0
58	Protein-free + cystine	0	7.4	3.85	0.80	40.4	9.3
59	Amino acids VI	93	12.2	4.06	0.92	43.6	8.7
60	Amino acids VI + methionine	126	16.6	4.20	0.77	44.5	8.7
61	Amino acids VI minus valine + methionine	118	9.0	3.78	0.78	48.1	8.7

amino acids plus some of the unessential amino acids noted in mixtures I and II. The plasma protein output rises in period 26 and reaches a miximum in period 27. There is also a gain in weight and a strong positive nitrogen balance.

Periods 28 and 30 show that the amino acid growth mixture *minus arginine*

is not well tolerated and not effective for plasma protein output. The mixture was not well taken. There was considerable vomiting and periods 28 and 30,

TABLE 4-*a*

Nitrogen Balance with Amino Acid Mixtures Obtained without Histidine and Arginine

Dog 40–122.

Period 7 day	Diet	Nitrogen balance							
		Intake		Output					Intake minus output
		In diet	In excess R.B.C. injected	In plasma	In feces	In urine			
						Total	Urea + NH_3	Undetermined	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*
23	Amino acids I	16.6	1.3	1.7	2.4	13.3	75.9	3.2	+0.5
24	Casein digest by mouth	17.5	0.4	3.2	2.7	11.3	76.1	2.9	+0.7
25	Amino acids II	17.8	2.6	1.0	1.8	14.0	76.8	3.3	+3.6
26	Amino acids III	17.9	4.0	1.8	1.2	10.8	73.8	2.8	+8.1
27	Amino acids III	17.9	0.2	2.6	1.8	9.7	66.9	3.2	+4.0
28	Amino acids IV	7.7	0.6	2.1	1.5	9.4	75.2	2.3	−4.7
29	Low protein	2.5	−1.6	0.3	2.4	6.0	61.9	2.3	−7.8
30	Amino acids IV	11.7	2.7	0.3	0.9	9.2	53.7	4.3	+4.0
31	Low protein	2.5	−1.5	0.3	1.8	6.1	65.6	2.1	−7.2
32	Casein digest by mouth	17.5	3.6	0.6	2.3	8.3	68.7	2.5	+9.9
33	Casein digest by mouth	17.5	−1.9	3.4	3.2	11.9	79.1	2.5	−2.9
34	Fasting	0.0	−1.8	0.4	0.0	7.8	66.7	2.6	−10.0
35	Amino acids IV + Liver	12.2	6.1	1.9	—	14.0	64.8	4.9	−0.6*
Totals		159.3	14.7	19.6	25.0	131.8			−2.4
56	Protein-free, 2 days + Pancreas, 5 days	24.1	5.6	2.1	4.1	12.4	80.3	2.5	+11.1
57	Pancreas	33.7	6.2	4.7	4.8	15.7	87.6	0.9	+14.7
58	Protein-free + cystine	1.1	0.3	1.2	2.4	6.2	51.4	3.0	−8.4
59	Amino acids VI	15.1	3.5	2.0	—	14.5	72.4	4.0	−0.9*
60	Amino acids VI + methionine	20.5	4.0	2.7	‡	19.3	—	—	+2.5
61	Amino acids VI minus valine + methionine	19.2	1.3	1.5	2.5	18.7	78.2	4.1	−2.2
Totals		113.7	20.9	14.2	16.8	86.8			+16.8

* Fecal nitrogen assumed to be 3.0 gm.

‡ Included in urine nitrogen.

therefore, are not a fair test. However, taken together with periods 6 and 7 in Table 1, they indicate clearly that arginine is essential for continued production of plasma protein.

Periods 29, 31, and 34 show again that a very low protein intake in a plasma depleted dog permits little or no removal of new plasma protein.

Periods 32 and 33—After a long period of depletion the first period (casein digest) shows a slow rise to the basic output level which subsequently will be maintained. It is of course possible that some of the casein digest taken in

TABLE 4-*b*

Plasma Protein Production Declines When Lysine, Histidine, and Arginine Are Omitted from Diet

Dog 40-122.

Period	Day	Diet	Plasma protein level	Plasma protein removed	Urinary nitrogen Total
	1941		*per cent*	*gm.*	*gm.*
	July 29	Amino acids I	4.05	2.61	
	" 30	" " "	3.99	2.72	
	" 31	" " "	4.58	2.94	2.78
	Aug. 1	" " "	4.20	4.07	
23	" 2	" " "	3.59	2.75	4.00
	" 3	" " "	—	0.00	Lost
	" 4	" " "	3.48	0.27	
	" 5	Casein digest	3.63	1.23	4.64
	" 6	" "	3.56	0.26	
	" 7	" "	3.76	0.23	4.45
	" 8	" "	3.76	0.24	
24	" 9	" "	4.16	0.50	2.65
	" 10	" "	—	0.00	
	" 11	" "	4.41	3.62	
	" 12	Amino acids II	4.21	3.86	4.17
	" 13	" " "	4.04	2.67	
	" 14	" " "	3.80	2.30	3.92
	" 15	" " "	3.64	0.29	
25	" 16	" " "	3.59	0.28	5.48
	" 17	" " "	—	0.00	
	" 18	" " "	3.83	0.20	
	" 19	Amino acids III	4.09	0.61	4.63

during the first period, 32, is used to repair tissue or for other emergencies that take precedence over the plasma protein program.

Periods 56 and 57 show the response to *pancreas* feeding—in general about 7 gm. pancreas are required to produce 1 gm. plasma protein, a much better potency than previously reported (14).

Period 58 shows that cystine under these conditions produces little new plasma protein: that removed was largely "carry over" from the pancreas

period. Possibly the character of amino acid make up of the preceding diet protein is an important factor in the "cystine effect"—compare period 20, Table 1 following liver diet.

Period 59 shows the growth mixture in which *cystine replaces methionine.* There is effective plasma protein production but some weight loss and less satisfactory clinical state, just as is shown in Table 1, periods 10 and 22.

Period 60 shows the effect of *replacing the methionine.* There is some increase in the plasma protein output and the appetite and clinical condition show distinct improvement, but the urinary nitrogen remains high in contrast to the low urinary nitrogen in Table 2-*a*, periods 25 and 26.

Period 61—*Valine* is removed from the growth mixture of amino acids with prompt *decline* in plasma protein output and appetite. Valine is evidently an essential component under these conditions.

Experimental History—Tables 4, 4-*a*, and 4-*b*. *Dog 40-122*, an adult female beagle hound observed during 22 weeks of protein digest experiments reported elsewhere (8) was fed amino acids I in the 23rd week. Spoon feeding was necessary after 3 days and 50 per cent of one diet was vomited. Vomiting continued the 1st day of period 24, losing 20 per cent, but thereafter the casein digest, 20 gm. daily, was eaten and retained. Basal diet B was given during periods 23 through 35 except for periods 29 and 31 with basal diet A.

Amino acids II was entirely spoon fed in period 25 as was amino acids III in periods 26 and 27. Erythema of the skin over joint prominences appeared with slight ulceration in three places. In other respects the dog was in good condition so that amino acids IV was tried. It was vomited completely on the 3rd, 5th, and 6th days and not offered on the 7th.

Basal diet A was consumed voluntarily, the dog was in good condition and showed no progression of the skin lesions. Amino acids IV was then again fed in period 30 and somewhat greater retention achieved, but by the 6th day spoon feeding was strongly resisted. Basal diet A was eaten voluntarily in period 31. By this time the skin ulcers were rather deep and appeared devoid of granulation tissue, but healing proceeded moderately well during the casein digest feeding periods 32 and 33. Fasting followed by a third trial of amino acids IV prompted a reappearance of the ulcers. Vomiting of the spoon fed mixture forced a change to pork liver, 50 gm. daily, for the last 3 days of period 35.

Following a series of digest infusion experiments (8), the dog showed the effects of protein strain or vitamin deficiency and had erythematous areas over the joints of the extremities with one ulcer. Fresh beef pancreas was fed uncooked in periods 56 and 57 along with the basal cake, 155 gm., during which time the skin condition improved. In period 58 *l*-cystine 1.0 gm. was added to the basal cake. During this and the remaining 3 periods choline chloride was increased to 0.4 gm. daily.

Amino acids V was spoon fed and retained for 3 days and, although some vomiting occurred on subsequent days, the net intake for the week was 77 per cent. The skin ulcers recurred by the end of this period. The fecal nitrogen collection was lost for this period.

In period 60, *dl*-methionine, 1.25 gm., was added daily to amino acids V and food

consumption was 100 per cent. Skin ulceration lessened, then remained stationary, but the general condition was improved over the preceding period. Several liquid stools contaminated the urine before additional bone ash took effect.

With valine omitted from the mixture of period 60 and glycine increased 1.0 gm., appetite remained good until the last day in this period 61; then spoon feeding afforded 100 per cent consumption. The skin ulcers remained unchanged and the dog appeared in fair clinical condition. The next experimental periods are reported elsewhere (8).

DISCUSSION

Methionine is found in the experiments reported here to be an essential amino acid for *long continued* plasma protein production and adequate body nutrition. Cystine substituting for methionine stimulates good plasma protein production for short periods (7 days), but other body processes suffer, as evidenced by loss of weight and a declining clinical condition. The presence of ample *choline* in the diet does not prevent this decline but the refeeding of methionine does. These observations are consistent with current information regarding the function, the relative importance, and the interrelation of methionine, cystine, and choline (22, 5). With such information, however, we are not able to explain how simple addition of cystine alone to a proteinless diet first stimulates plasma protein formation, then loses its effect, and then regains it when all essential amino acids *except methionine* are added to the diet, as is the case in periods 19 to 22, Table 1. Cystine under certain conditions appears to act as a stimulant in the reaction leading to plasma protein production.

The observations on plasma protein metabolism reported from this laboratory have a bearing on total body nitrogen metabolism. The experiments here reported with mixtures of amino acids contribute additional information. We are tempted to say a word on the controversial subject of *exogenous* and *endogenous* nitrogen metabolism.

The theories of protein metabolism of Voit and of Pflüger of the last century were questioned by Folin's reasoning from analysis of urinary constituents (4). Because he found in 1905 that the absolute excreted quantities of creatinine and uric acid and neutral sulfur remained relatively constant through wide variations in intake of diet protein, he concluded that these end products were a measure of those metabolic processes "indispensable for the continuation of life" and called this total the *constant* metabolism, the *tissue* metabolism, or the *endogenous* metabolism. The "variable protein metabolism," as measured most strikingly by the urea and the ammonia fractions and the inorganic sulfate excretion, he called the *exogenous* metabolism. It is of interest that he admitted the possibility that those measures of exogenous metabolism, urea, etc., might to some extent represent the endogenous metabolism. But he believed that it would be possible to plan feeding experiments which would

greatly reduce the urea fraction below his then low figure (near 60 per cent) and held fast to his belief in *two distinct kinds* of protein metabolism.

That there might be a fairly rapid flow of protein from plasma into the tissues was first demonstrated in this laboratory in 1934 (6), when it was found that homologous plasma protein given by vein supplied all body needs for protein. That a certain fraction of tissue protein (called *reserve store*) can be readily converted into plasma protein has been frequently demonstrated. It was postulated that the relationship existing between the plasma proteins and the tissue proteins was a *dynamic* equilibrium and that the demands of the moment through the forces exerted would determine the direction of protein flow. Plasma protein might be made rapidly from tissue protein following hemorrhage, or tissue protein might be furnished rapidly by plasma protein during protein starvation. It was easily demonstrated that diet protein nitrogen was rapidly converted into new plasma protein. The total body protein, therefore, appeared to be in a *dynamic steady state* involving diet proteins, tissue proteins, and plasma proteins.

The dynamic theory met opposition (20, 19) and then strong support (17). Feeding amino acids labeled with heavy nitrogen, Schoenheimer and his colleagues found a most rapid exchange between dietary nitrogen and plasma protein and tissue protein nitrogens. So quickly did dietary nitrogen appear in tissue proteins and in such common measures of endogenous metabolism as urinary creatinine (1) that these workers were inclined to question the existence of any real difference between endogenous and exogenous metabolism. Other workers, notably Burroughs, Burroughs, and Mitchell (2), have upheld the distinction.

The concept of *two separate types* of nitrogen catabolism must be discarded if the term endogenous is taken to exclude recent dietary nitrogen as a source, for it is clear that a large fraction of urinary creatinine may arise in creatine formed from recently ingested protein (1). Folin did not envisage this possibility; otherwise he would not have chosen the terms endogenous and exogenous. If there is a whole group of reactions of a relatively constant and irreversible character such as the one involving synthesis and degradation of creatine, as is suggested by Mitchell (13) to represent an "independent endogenous metabolism," then this group of reactions needs only a more appropriate designation. However, until it is demonstrated that an animal can exist independent of those hydrolytic, interconversion, and transamination reactions which appear to account for the greater portion of intermediate protein metabolism, it may be wise to discard the concept of two independent types of metabolism.

It appears more likely that total metabolism consists of many *interdependent* reactions possibly divisible into two *interdependent* groups. As described by Mitchell, the one may be distinguished by its relative constancy and by a

certain irreversibility. But that it may be regarded as independent of the other more variable group appears very unlikely. The level of protein metabolism can be greatly reduced below normal but we do not believe that those reactions characteristic of the variable fraction can be eliminated. They are merely reduced to a minimum. The *minimal* or *basal* nitrogen metabolism consists of the sum of this minimum of the more *variable* fraction plus the more *constant* fraction.

The *minimal nitrogen metabolism* thus includes all reactions and reaction materials present in normal or maximal metabolism functioning at minimum rates. As Schoenheimer's experiments indicate, "all reactions, for which specific enzymes and substrates exist in the animal, are carried out continually;" each reaction merely has its limits of rate and of mass. The minimal nitrogen catabolism is a fairly definite and constant quantity for each animal and species, as well demonstrated by Mitchell and by others (13), and cannot be further depressed even by the feeding of selected amino acid mixtures (2, and Tables 1-*a* and 2-*a* above).

There appears to be a close parallelism between minimum nitrogen metabolism and depletion of protein reserve stores. Catabolism of reserve protein is probably an important source of the more variable excretion products—urea, etc. Complete catabolism of reserve protein or complete depletion of it, as by plasmapheresis, is not possible. In those dogs which we have called "stripped of their reserve stores of protein" (10) small reserves are probably always present, continually being utilized for maintenance and replacement. These stores are reduced merely to the minimum, urea and ammonia production is reduced to the minimum, as is total nitrogen catabolism, and *new plasma protein production* is reduced likewise to its minimum. We have said that little new plasma protein is formed by the unfed depleted dog but we appreciate that more may be produced and go unrecognized because it replaces plasma protein lost into the tissues—a normal maintenance and replacement fraction however small.

In summary, the broad view of protein metabolism indicated by the evidence today should recognize these principles:—

1. Exogenous nitrogen enters rapidly into most internal reactions of body nitrogen metabolism.

2. There are obvious differences between these reactions but none has independent existence. All are interwoven into a dynamic steady state.

3. All reactions in internal nitrogen metabolism continue all of the time, each with its characteristic rate and mass limitations. The algebraic sum of the minimum rates of all these reactions constitutes a definite *minimum nitrogen metabolism.*

4. All differences in normal total protein metabolism are differences of quantity not of kind.

These observations in dogs should provoke early clinical trial of similar mixtures of pure amino acids. The value of *plasma proteins* in nutrition and resistance to disease as well as in shock is no longer a matter of debate. Plasma protein (as plasma) alone given by vein can supply all the protein requirements of the body over considerable periods.

If the body can be supplied with non-toxic material by vein out of which new plasma protein can be produced, this material may be used to supplement human plasma, the supply of which is limited. The ideal solution of amino acids for intravenous use would seem to be a mixture of the essential pure crystalline amino acids. To judge from the experimental findings, this mixture could be used with security by vein in considerable amounts, replacing or supplementing saline or glucose solutions. In this way the protein requirements, at least in part, might be met and repair of injured tissues facilitated. This amino acid solution might prove effective in many conditions in which protein is required yet in which it cannot be given adequately by mouth, for example, in burns, alimentary tract injuries or abnormalities, peritonitis, and certain liver injuries. The amino acid complex may prove valuable in the treatment of shock in the initial stages as well as during the later recovery period, not as a plasma substitute but as an adjunct to the nutritive and tissue protein restoring function of the plasma. Plasma may successfully combat immediate shock, but eventual recovery may depend on how well and how early the lost tissue protein nitrogen is replaced. Furthermore, new plasma protein should be produced by the amino acid mixture, and perhaps it will reduce or eliminate the need for further plasma injections on the days following the shock-producing injury. One very limited clinical trial of a mixture of pure amino acids has been reported (18).

A *substitute for plasma* given by vein is not yet available, but there is reasonable hope that adequate substitutes can be found to replace much of the plasma now used and to supplement plasma whenever given. Non-toxic protein digests supplemented by amino acids hold much promise, but a mixture of amino acids in the future may be most valuable because of stability, concentration, ease of use, and lack of toxicity. Proteins from animal sources always will carry the danger of intoxication for certain human beings. One can look forward with confidence to rapid progress in this important field.

SUMMARY

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

When the protein intake of such dogs is completely replaced by the growth

mixture (Rose) of crystalline amino acids, plasma protein production is excellent, weight and nitrogen balance are maintained. This growth mixture consists of ten amino acids, threonine, valine, leucine, isoleucine, tryptophane, lysine, phenylalanine, methionine, histidine, arginine, and is as effective as most diet proteins in plasma protein production.

The above amino acid mixture in aqueous solution may be given by vein with equally good plasma protein production and no apparent clinical disturbance even when given rapidly.

Cystine may replace methionine in the above mixture with equally good plasma protein production for 7 to 10 days but at the expense of the body tissues, that is, with weight loss and a negative nitrogen balance.

The addition of cystine to the protein-free, otherwise adequate diet may result in the production of considerable new plasma protein during a period as long as 1 week (cystine effect). This reaction may depend upon the amino acid constitution of the preceding diet protein in that it occurred following a liver feeding but did not occur after pancreas feeding.

Arginine is required in the diet of the protein depleted dog for fabrication of plasma protein. It is apparently not needed for nitrogen balance for as long as 1 or 2 weeks.

The omission of either threonine or valine from the growth mixture is quickly followed by a sharp decline in plasma protein formation and by a negative nitrogen balance.

When histidine, arginine, and most of the lysine are omitted from the growth mixture, nitrogen balance and weight may be maintained for as long as 1 week but plasma protein production falls off markedly.

The findings indicate that the growth mixture of amino acids should be a valuable addition to transfusion and infusion therapy in disease states associated with deficient nitrogen intake or tissue injury and accelerated nitrogen loss, including shock, burns, and major operative procedures.

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