AMINO ACID MIXTURES EFFECTIVE PARENTERALLY FOR LONG CONTINUED PLASMA PROTEIN PRODUCTION. CASEIN DIGESTS COMPARED*‡

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Our objective in the experiments tabulated below is a better understanding of the amino acid requirements for plasma protein production. It is important to determine the optimal make-up of mixtures which are used by the plasma-depleted dog to produce *new plasma proteins*. Briefly it may be said that the better mixtures of amino acids rate with the best food proteins in the production of new plasma protein in dogs standardized as described below under Methods. They are better tolerated when given intravenously than are casein digests, producing no clinical disturbance when given several times faster than the tolerable rate for such digests. They provide nitrogen balance as well as plasma protein production over periods of several months.

In a previous report (11) it was found that good plasma protein regeneration was obtained from feeding or injecting the ten amino acids determined by Rose (21) to be essential for the growth of rats. Further tests have now been made of various mixtures of these ten acids and of non-essential amino acids. Similar mixtures of the ten essential amino acids plus glycine have appeared similarly well used and tolerated in the first trial in a human patient recently reported (2) as well as in other trials in patients to be reported soon. These mixtures appear not to have the undesirable vomiting and pyrogenic effects noted in an earlier report regarding the use in infants of an amino acid mixture containing more of the non-essential amino acids (25).

Subcutaneous administration of the better mixtures of amino acids in 10 per cent solution in distilled water produces no clinical disturbance in dogs or in man and is accompanied by good utilization. The availability of the subcutaneous route is a great boon in many instances where repeated daily injections are indicated. It is particularly fortunate that solutions of high concentration are so well tolerated.

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Methods

These experiments follow the same general pattern of those in previous reports (11, 12). A dog is standardized for tests of plasma protein production by a process of body protein and plasma protein depletion. The depletion is accomplished by diet restriction and plasmapheresis. A depleted state is reached in which the kind or the quantity of the diet intake is found to be fairly directly responsible for the size of the plasma protein removal (by plasmapheresis) necessary to maintain the depleted level. Thus the dietary nitrogen is said to have yielded the plasma protein nitrogen. If the diet and its nitrogen are of good quality and amount they will provide during periods

TABLE 1

Amino Acid Mixtures

	Amounts given daily										
	Vg	Vj	Vm	Vo	Vt	Vy	Vaa	Vab	Vac	Vad	Vae
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
dl-Threonine	0.7	0.7	1.0	1.0	0.7	2.0	0.7	0.7	1.4	1.4	1.4
dl-Valine	1.5	1.5	1.5	1.5	1.5	3.0	1.5	1.5	1.5	1.5	1.5
dl-Leucine	3.0	3.0	3.0	3.0			2.2				
l (−)-Leucine					1.5	2.2		1.1	2.2	2.2	2.2
dl-Isoleucine	1.4	1.4	1.4	1.4	1.4	2.0	1.0	1.0	1.0	2.0	2.0
dl-Lysine·HCl	1.5	1.5	1.5	1.5	1.5						
l (+)-Lysine·HCl						3.0	1.1	1.1	1.1	1.1	1.1
dl-Tryptophane							0.3	0.3	0.3	0.6	0.3
l (−)-Tryptophane	0.4	0.4	0.5	0.5	0.4	0.6					
dl-Phenylalanine	1.0	1.0	1.5	3.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0
dl-Methionine	0.6	0.6	0.8	0.8	0.6	1.3	0.6	0.6	0.6	0.6	0.6
l (+)-Histidine·HCl	0.5	0.5	0.5	0.5	0.5	1.3	0.5	0.5	0.5	1.0	1.0
l (+)-Arginine·HCl	0.5	0.5	0.8	0.8	0.5	1.5	0.5	0.5	1.0	1.0	1.0
Glycine	6.8	1.0	1.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0
Totals	17.9	12.1	13.5	15.0	10.6	20.9	10.4	9.3	11.6	13.4	13.1

of many months all body nitrogen requirements for maintenance and for considerable production of new plasma protein. If the nitrogen intake is of deficient quality or amount, the body pool of protein nitrogen will be lowered and plasma protein production decreased.

Plasmapheresis as used in these experiments means daily bleeding of suitable amounts followed immediately by return of red blood cells centrifuged from the blood of normal donor dogs and resuspended in a saline solution. In all plasmapheresis experiments previously reported from this laboratory, the red cells returned were washed once in saline. The amount of plasma nitrogen retained by centrifuged red cells from which the supernatant plasma had been carefully siphoned was recently measured and found too insignificant to warrant the additional handling of the cells. *Diet restriction* during protein depletion typically means a proteinless diet for the first 2 weeks and then 1+ gm. protein per kilo and 80 calories per kilo with ample vitamin and mineral accessories, if possible. A *standard protein-depleted dog* is one subjected to plasmapheresis during diet control over a long enough period for constant relationship to be demonstrated between the measured diet nitrogen intake and the measured plasma protein nitrogen output while at the same time weight and nitrogen equilibrium are being maintained and the blood plasma protein level is being kept at a steady low level. A dog so standardized appears clinically normal.

The *various mixtures of crystalline amino acids* used are listed in Tables 1 and 1-*a*. When given orally, they were mixed in the basal diet as such. When given paren-

TABLE 1-*a*

Amino Acid Mixtures

	Amounts given daily							
	Vd	Va	Ve	Vf	Vda	VIII	Vk	IX
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
dl-Threonine	0.7	1.3	0.7	0.7	0.7	0.7	0.7	1.5
dl-Valine	1.5	1.5	1.5	1.5	1.5	1.5	1.5	2.3
dl-Leucine			3.0	3.0	3.0	3.0	3.0	4.5
l(−)-Leucine	1.5	1.5						
dl-Isoleucine	1.4	1.4	1.4	1.4	1.4	1.4	1.4	2.1
dl-Lysine·HCl	1.5	3.0	3.0	1.5	1.5	1.5	1.5	2.3
l(−)-Tryptophane	0.2	0.4	0.4	0.4	0.2	0.6	0.6	0.8
dl-Phenylalanine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.3
dl-Methionine	0.6	1.2	1.2	1.2	0.6	0.6	0.6	1.2
l(+)-Histidine·HCl	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.8
l(+)-Arginine·HCl	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.2
Glycine	7.6	5.4	5.0	6.5	6.7	5.8	6.5	1.0
dl-Alanine								0.8
dl-Glutamic acid								2.0
l(−)-Cystine						0.5		0.5
l(−)-Tyrosine						1.0		0.5
l(−)-Proline								0.5
Totals	17.0	17.7	18.2	18.2	17.6	18.6	17.8	24.3

terally, they were prepared in fresh solution daily by dissolving in recently boiled hot, distilled water followed by filtration through hard paper. The Amigen, an enzymatic digest of casein and pork pancreas, was kindly furnished by Mead Johnson and Company in 10 per cent solution. "Amino Acids Stearns" a fortified acid hydrolysate of casein, was kindly furnished by Fredrick Stearns and Company in 15 per cent solution.

The *diet compositions* other than the amino acids are detailed here but the amount and the time of their administration is given in the Experimental History of each dog. The *proteinless 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 1.6, salt mixture (14) 1.2, bone ash 4.0, kaolin 4.0, and water 9.6, all baked into a dry cake containing 4.9 calories per gm.

Diet H 30 was an uncooked mixture of dextrose 70, corn oil 6, Crisco 12, cod liver oil 2, yeast powder (Standard Brands, Inc., No. 200-B) 2, liver powder (Eli Lilly and Company, liver fraction rich in B_2 complex) 2, salt mixture (14) 3, bone ash 3. *Diet H 31* was diet H 30 with yeast and liver powders omitted.

The vitamin capsules and the vitamin emulsion were very kindly prepared and furnished by Eli Lilly and Company.

	Capsules Daily unit	Emulsion 10 cc.
Vitamin A, U.S.P. units	5000	5000
Thiamin chloride	3 mg.	6 mg.
Riboflavin	2 mg.	6 mg.
Pyridoxin hydrochloride	1 mg.	5 mg.
Calcium pantothenate	1 mg.	5 mg.
Nicotinamide	20 mg.	50 mg.
Ascorbic acid	50 mg.	50 mg.
Vitamin D, U.S.P. units	500	500
Mixed natural tocopherols	50 mg.	50 mg.
Choline chloride	100 mg.	100 mg.
2-methyl-1,4-naphthoquinone	1 mg.	
Rice polish concentrate	1000 mg.	1000 mg.

Aseptic and antiseptic technique was employed as far as possible. Macro Kjeldahl nitrogen determinations were done in duplicate on all intake and output specimens.

Parenteral injections of amino acids mixtures or protein digests were made into the leg veins or subcutaneously or intraperitoneally as noted in the tables and in the Experimental History, Dog 41-187. Amino acid mixtures intravenously were usually given in about 200 cc. solution and in 30 minutes time. Some variations in concentration and in speed of injection are mentioned in the Experimental Observations and others will be described in a later report. The Amigen and the "Amino Acids Stearns" were given at slower rates as described in Experimental History, Dog 41-187.

EXPERIMENTAL OBSERVATIONS

The data on plasma protein production and nitrogen balance obtained from two dogs by long continued amino acid feeding and injection are recorded in table form. Tables 2 and 2-*a*, 3 and 3-*a* present the data of Dog 41-187 for 33 consecutive weekly periods. Tables 4 and 4-*a* are similarly constructed for 25 weeks continuous observation of Dog 41-122. The various mixtures of amino acids tested are listed in Tables 1 and 1-*a*.

Amino Acid Injection Experiments.—(Tables 2 and 2-*a*, 3 and 3-*a*). For 30 consecutive weeks, (210 days), Dog 41-187 was maintained in nitrogen and weight balance and produced considerable blood plasma protein from amino acids injected intravenously, subcutaneously, or intraperitoneally. The amount of plasma protein produced was comparable to that which might be obtained from feeding any one of several good proteins by mouth (12). Expressed in terms of *plasma protein nitrogen removed as per cent of amino acid nitrogen injected values of more than 20 per cent* are obtained for the more efficient amino acid mixtures tested. During all of these 33 weeks the dog was in good clinical condition except for the evidences of slight vitamin deficiency described in the Experimental History below. There was no toxic reaction to any of the more than 150 injections of the amino acid synthetic mixtures except during periods 13 to 15 when deliberate attempts to test the upper limits of tolerance were made. On some days a total intake of more than 20 gm. amino acids was injected within 20 minutes. Much slower injections of protein digests produced clinical disturbance, chiefly vomiting.

Periods 1 to 3 (Tables 2 and 2-*a*) were devoted to the standardization of the test animal by the process of *protein depletion* as described under Methods. Of the 76.5 gm. plasma protein removed about 15 gm. may be ascribed to the liver diet of period 2. This leaves 61.5 gm. plus at least 5 gm. carry over into period 4 minus about 6.5 gm. accountable as a reduced circulating mass of plasma protein, a net total of 60 gm. to be explained as plasma protein produced from reserve tissue proteins during the first 4 weeks of depletion.

Periods 5 to 12 (Tables 2 and 2-*a*) show weekly *plasma protein production* generally maintained above 15 gm. with limits of 11 to 19 gm. The changes in amino acid mixture (see Tables 1 and 1-*a*) produced no consistent change in plasma production so that the Vj mixture should be considered the most efficient, having the lowest total content. The Vt mixture of *periods 16 to 20* is equally if not more efficient but like the Vj mixture is associated with a slight weight loss. When the original Vt was doubled in periods 18 and 19, weight gain was recorded and plasma protein production was increasing.

Periods 13 to 15 (Tables 2 and 2-*a*) were devoted to tests of tolerance to the *very rapid injection of various mixtures of amino acids* the results of which will be reported later. It may be mentioned that of the 21 daily injections, 9 consisting of mixtures of the ten essential amino acids *and glycine*, were given at rates from 6 to 14 mg. nitrogen per minute per kilo *without reaction* and 8 consisting of mixtures of the ten essential amino acids without addition of glycine were given at rates from 4 to 12 mg. nitrogen per minute per kilo with vomiting. On two occasions without glycine there was no vomiting and on one with glycine there was vomiting. Once a glycine containing mixture was given slowly (3 mg. nitrogen per minute per kilo) with the expected lack of reaction.

During periods 21 to 25 (Tables 3 and 3-*a*) *protein digests* failed to perform as well as the amino acid mixtures. Despite slower injection rates (2 to 5 mg. nitrogen per minute per kilo) a little less plasma protein was produced per gram of nitrogen intake than was produced from amino acids during periods 13 to 15 (Tables 2 and 2-*a*). In addition the slower rate of injection gave no better nitrogen retention from the standpoint of the ratio of urinary nitrogen output to injected nitrogen intake—0.75 in each instance. And at this slower rate, vomiting occurred during 8 of 14 injections, 7 of which were given 2 to 4 mg.

nitrogen per minute per kilo. The poorer plasma protein production with *Amigen* may well be related to the clinical disturbance associated with vomiting. Production was poorer during periods 13 to 15 (Tables 3 and 3-*a*) when combinations of amino acids which induce vomiting were given than during the

TABLE 2

Plasma Protein Production with Amino Acid Mixtures Given Intravenously, Subcutaneously, or Intraperitoneally

Dog 41-187

Period 7 days	Diet (Amino acid mixtures Vg, Vj, etc., are defined in Table 1)	Intake N × 6.25 Total for 7 days		Plasma protein removed Total for 7 days	Blood plasma average concentration		R.B.C. hematocrit, average	Weight
		Amino acids	Basal		Total protein	A/G ratio		
		gm.	*gm.*	*gm.*	*per cent*		*per cent*	*kg.*
	Initial				5.35	0.94	50.0	12.8
1	Protein-free	0	2	27.6	4.27	—	51.9	12.7
2	Liver	0	72	28.4	4.38	0.92	49.8	12.5
3	Protein-free	0	2	20.5	4.30	0.67	47.5	12.4
4	Vg, i.v. (Va with ½ threonine, lysine, methionine)	117	2	23.5	4.14	0.73	49.2	11.9
5	Vg, i.v.	117	2	18.1	3.85	0.84	49.3	11.8
6	Vg, i.v.	117	2	14.6	3.90	1.06	49.2	11.5
7	Vg, s.c.	117	2	12.9	3.91	1.12	51.3	11.8
8	Vj, s.c. (Vg with less glycine)	69	2	15.0	3.98	1.03	52.5	11.7
9	Vj, s.c.	69	2	17.0	3.89	1.14	50.5	11.6
10	Vm, s.c. (Vj with increased threonine, tryptophane, phenylalanine, methionine, arginine	78	2	11.0	3.82	1.00	48.7	11.7
11	Vm, i.p.	78	2	18.6	4.02	1.03	52.2	11.3
12	Vo, s.c. (Vm with phenylalanine doubled)	83	2	16.3	3.92	0.99	54.1	11.1
13	Varied mixtures, i.v.	138	2	17.3	3.96	1.02	47.7	10.6
14	Varied mixtures, i.v.	107	19	14.6	3.94	1.12	47.4	10.8
15	Varied mixtures, i.v.	122	19	17.7	4.01	1.08	48.0	10.5

other periods of amino acid injection in Tables 2 and 2-*a*, 3 and 3-*a*, when mixtures were used which do not cause vomiting despite rapid injection.

"Amino Acids Stearns" was less well tolerated than Amigen. Moreover, *the urinary nitrogen was greater than the digest nitrogen intake*, and after the carry-over effect had worn off in period 23 *virtually no plasma protein was produced* in period 24 (Tables 3 and 3-*a*). During period 25, in order to determine whether or not improvement in utilization might be obtained by supplementation of "Amino Acids Stearns," a half quantity of Vj mixture was added to a half quantity of "Amino Acids Stearns," but the attempt was discontinued on account of the poor tolerance described in the Experimental History.

In periods 26 to 31 (Tables 3 and 3-*a*) mixture Vaa and its variants Vab and Vac appear to be even more efficient than Vj or Vt, but here again, we believe,

TABLE 2-*a*

Positive Nitrogen Balance with Amino Acid Mixtures Given Parenterally

Dog 41-187

Period 7 days	Diet (Amino acid mixtures Vg, Vj, etc., are defined in Table 1)	Nitrogen balance						
		Intake			Output			Intake minus output
		In diet		In excess R.B.C. injected	In plasma	In feces	In urine	
		Amino acids	Basal					
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
	Initial							
1	Protein-free	0	0.4	0.7	3.5	0.6	8.8	−11.8
2	Liver	0	11.6	2.6	4.6	2.8	9.0	−2.2
3	Protein-free	0	0.4	−0.5	3.3	0.9	11.8	−16.1
4	Vg, i.v. (Va with ½ threonine, lysine, methionine)	18.7	0.3	5.3	3.8	1.3	15.4	+3.8
5	Vg, i.v.	18.7	0.3	3.6	2.9	1.5	14.4	+3.8
6	Vg, i.v.	18.7	0.3	0.6	2.4	1.0	14.6	+1.6
7	Vg, s.c.	18.7	0.3	9.1	2.1	0.7	12.0	+13.3
8	Vj, s.c. (Vg with less glycine)	11.1	0.3	−1.5	2.4	1.3	7.2	−1.0
9	Vj, s.c.	11.1	0.3	−1.3	2.8	1.1	6.6	−0.4
10	Vm, s.c. (Vj with increased threonine, tryptophane, phenylalanine, methionine, arginine)	12.4	0.3	2.6	1.8	1.7	6.8	+5.0
11	Vm, i.p.	12.4	0.3	2.6	3.0	1.8	10.2	+0.3
12	Vo, s.c. (Vm with phenylalanine doubled)	13.3	0.3	2.1	2.7	1.3	8.9	+2.8
13	Varied mixtures, i.v.	18.2	0.3	3.5	2.9	0.9	10.8	+7.4
14	Varied mixtures, i.v.	17.1	3.0	4.5	2.3	1.1	15.9	+5.3
15	Varied mixtures, i.v.	19.5	3.0	5.4	2.9	1.9	14.4	+8.7
	Totals	189.9	21.4	39.3	43.4	19.9	166.8	+20.5

and recent experiments appear to confirm that this mixture is short of the optimal quantity for this dog over long periods. The actual production of periods 28 to 31 steadily diminishes from a high point probably reached as the result of the cumulative effect of 2 weeks on the very ample mixture Vy, periods 26 and 27.

During periods 32 and 33 (Tables 3 and 3-*a*) the decline in protein production is registering the effects of the prolonged period on the inadequate and incomplete synthetic vitamin supply. Some of the other effects of this deficiency are presented in the Experimental History.

TABLE 3

Plasma Protein Production Amino Acid Mixtures Superior to Certain Casein Digests

Dog 41-187

Period 7 days	Diet (Amino acid mixtures Vt, etc., are defined in Table 1)	Intake N × 6.25 Total for 7 days		Plasma protein removed Total for 7 days	Blood plasma average concentration		R.B.C. hematocrit, average	Weight
		Amino acids	Basal		Total protein	A/G ratio		
		gm.	*gm.*	*gm.*	*per cent*		*per cent*	*kg.*
16	Vt, s.c. (Vj with $l(-)$-leucine)	62	33	17.0	4.12	0.84	48.3	10.6
17	Vt, s.c.	62	33	17.2	4.02	0.85	48.4	10.6
18	Vt, s.c.	124	33	18.0	4.09	0.79	47.4	10.9
19	Vt, s.c.	124	2	21.9	4.01	0.85	46.8	11.1
20	Vt, s.c.	62	2	14.9	3.80	0.75	46.9	10.7
21	Amigen, i.v.	134	31	12.6	3.97	0.52	47.2	11.2
22	Amigen, i.v.	134	31	14.2	4.08	0.55	47.1	10.9
23	Amino Acids Stearns, i.v.	139	21	13.9	3.87	0.53	47.1	11.4
24	Amino Acids Stearns, i.v.	139	31	1.6	3.73	0.70	45.6	11.5
25	Vj, i.v. and s.c. Amino Acids Stearns, i.v.	89	31	11.6	3.95	0.80	43.6	11.7
26	Vy, s.c.	128	21	19.7	4.12	0.98	47.6	11.9
27	Vy, s.c.	128	31	18.1	3.96	0.90	48.2	12.5
28	Vaa, s.c.	61	31	25.2	4.37	0.90	48.1	12.1
29	Vaa, s.c.	61	0	21.0	3.93	0.73	50.0	12.3
30	Vaa-Vab, s.c. (Vaa with $l(-)$-leucine)	55	0	19.8	3.98	0.64	49.3	11.9
31	Vac, s.c. (Vab with double threonine, leucine, arginine)	70	0	17.8	3.93	0.58	48.8	11.7
32	Vad, s.c. (Vac double isoleucine, tryptophane, histidine)	81	0	13.4	3.95	0.59	48.8	11.4
33	Vae, s.c. (Vad with ½ tryptophane)	79	0	9.9	3.99	0.59	49.5	11.3

The large positive nitrogen balance for the 33 periods in Dog 41-187 of more than 95 gm. suggests a method error but we have detected none. We realize that nitrogen can be deposited even while weight is being lost, but to deposit a quantity equal to one-fourth of the approximate total body nitrogen without gaining weight appears very unusual. Nitrogen losses as desquamated skin, hair, and materials from ulcer surfaces were not measured, but how much this omission contributes to the abnormally high positive balance we do not know. Certainly the low urinary nitrogen levels of such periods as 8, 9, 10, 16, and 17 suggest very good utilization of the injected amino acids. Data previously presented and discussed (11) suggest that 4.5 gm. is the approximate urinary nitrogen output of the *minimal nitrogen metabolism* of this dog.

TABLE 3-*a*

Positive Nitrogen Balance with Amino Acid Mixtures and Certain Casein Digests Given Parenterally

Dog 41-187

Period 7 days	Diet (Amino acid mixtures Vt, etc., are defined in Table 1)	Nitrogen balance						
		Intake			Output			Intake minus output
		In diet		In excess R.B.C. injected	In plasma	In feces	In urine	
		Amino acids	Basal					
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
16	Vt, s.c. (Vj with *l*(−)-leucine)	10.0	5.2	0.3	2.8	1.9	6.9	+3.9
17	Vt, s.c.	10.0	5.2	2.6	2.8	1.6	5.4	+8.0
18	Vt, s.c.	20.0	5.2	2.6	2.9	1.4	14.0	+9.5
19	Vt, s.c.	20.0	0.3	−0.3	3.6	1.4	13.5	+1.5
20	Vt, s.c.	10.0	0.3	1.1	2.4	1.4	7.0	+0.6
21	Amigen, i.v.	21.5	5.0	0.8	1.9	0.6	14.8	+10.0
22	Amigen, i.v.	21.5	5.0	3.6	2.3	0.6	17.4	+9.8
23	Amino Acids Stearns, i.v.	22.3	5.0	2.9	2.2	0.6	22.9	+4.5
24	Amino Acids Stearns, i.v.	22.3	5.0	−2.1	0.3	0.5	23.4	+1.0
25	Vj, i.v. and s.c. Amino acids Stearns, i.v.	14.3	5.0	3.2	1.9	0.6	11.8	+8.2
26	Vy, s.c.	20.5	5.0	5.4	3.2	0.5	14.6	+12.6
27	Vy, s.c.	20.5	5.0	0.5	2.9	0.6	14.8	+7.7
28	Vaa, s.c.	9.7	5.0	2.7	4.1	1.2	10.4	+1.7
29	Vaa, s.c.	9.7	0	0.0	3.4	0.8	8.6	−3.1
30	Vaa-Vab, s.c. (Vaa with *l*(−)-leucine)	8.8	0	−0.2	3.2	0.5	7.9	−3.0
31	Vac, s.c. (Vab with double threonine, leucine, arginine)	11.2	0	2.2	2.9	0.7	11.9	−2.1
32	Vad, s.c. (Vac double isoleucine, tryptophane, histidine)	13.0	0	0.3	2.2	0.7	8.7	+1.7
33	Vae, s.c. (Vad with ½ tryptophane)	12.7	0	0.6	1.6	0.7	8.6	+2.4
	Totals	278.0	56.2	26.2	46.6	16.3	222.6	+74.9

Experimental History—Tables 2, 2-*a*, 3, and 3-*a*.

Dog 41-187, an adult female mongrel hound with medium-long hair was begun on a program of plasma depletion after 11 weeks rest from a similar experiment previously reported (11). During this rest period the dog gained weight from 8.8 kilos to 14.1 kilos. Fasting for a week was followed by a protein-free diet with vitamin supplements for one week and with pork liver, 50 gm. daily, added for 2 more weeks. The protein concentration of the plasma had fallen by this time to 5.35 gm. per cent and the weight to 12.8 kilos. Except for the low plasma protein the dog was in excellent clinical condition, slightly over-nourished in appearance with a good sleek coat of hair.

In period 1 the daily diet consisted of proteinless cake, 200 gm., and one unit of vitamin capsules as described in Methods.

In period 2 raw pork liver, 50 gm., was added to the diet of period 1.

In period 3 the diet of period 1 was resumed. From this period on the dog refused to eat voluntarily but cooperated very well with spoon feeding.

In period 4 the proteinless cake was reduced to 150 gm. daily and intravenous amino acids were begun. Daily injections into leg veins were done in 20 to 50 minutes with never a sign of reaction, and subcutaneous injections, begun in period 7, required similar periods. Subcutaneous injections were made over the abdomen, chest, or back. The subcutaneous space became more easily distended as time went on and the solution tended to travel to the dependent area. Absorption occupied several hours but no sign of irritation was ever observed.

In period 11 the intraperitoneal injections of amino acid solutions were done under pressure within 10 minutes or less. There was slight transitory discomfort upon some injections but no sign of persistent irritation.

In periods 13, 14, and 15 tolerance for the very rapid injection of various amino acid mixtures was tested. These results will be included in a separate report.

By period 13 skin changes similar to those previously noted (11) were again evident. Desquamation of hair and of epidermal layers continued gradually until weeping red areas appeared, chiefly over bony prominences, and proceeded to frank ulceration. Vitamin emulsion MH 42-11-91, 10 cc. daily, was substituted for the vitamin capsules.

In period 14 lard, 25 gm. and liver powder, 4 gm. were added to the daily diet.

In period 16 yeast powder, 4 gm., was added and the vitamin emulsion was omitted.

In period 19 the liver and the yeast powders were replaced by the vitamin emulsion, 10 cc. daily. The skin lesions continued to grow worse.

In period 21 the first injection of Amigen produced panting, salivation, vomiting, and defecation when 125 cc. of the 10 per cent solution was injected in 30 minutes. Vomiting occurred again during the injection of the remaining 125 cc. in 60 minutes. Vomiting occurred during 7 of the remaining 13 injections of periods 21 and 22, each of which was given within 75 to 180 minutes.

In period 23 "Amino Acids Stearns," 150 cc. diluted with distilled water to 250 cc., was given daily. After 50 per cent of the first injection had been given within 60 minutes the dog vomited and defecated and vomited 3 times more while the remaining 50 per cent was given within 45 minutes. Vomiting occurred frequently during the remaining 13 injections even when the injection was as slowly given as 210 minutes.

In period 25 "Amino Acids Stearns," 75 cc. and amino acid mixture Vj were made up to 300 cc. and given intravenously. In the 1st day the combination was given within 100 minutes with salivation, urination, and defecation but no vomiting. On the 2nd day it was given within 75 minutes with vomiting. For the rest of the period mixture Vj was given alone subcutaneously.

During periods 21 to 28 diet H 30, 210 gm., and the vitamin emulsion, 5 cc., constituted the basal intake. The skin lesions remained but did not appear to progress.

During periods 29 to 33 diet H 31, 210 gm., replaced diet H 30. There may have been slight progression of the skin lesions and since the plasma showed jaundice during period 33 (icterus index 3.5), plasmapheresis was discontinued. It should be mentioned that for the last 13 weeks the choline intake was at the low level of 50 mg. daily and impaired liver function might be expected (9).

The dog was observed for the next 15 weeks while on a dietary intake of 25 gm. commercial casein, 210 gm. H 31, and 5 cc. vitamin emulsion. After 3 weeks the plasma proteins were 5.23 per cent, icterus index less than 1, weight 11.2 kilos, and the skin lesions very slightly improved. After 6 weeks the lesions were obviously healing, plasma proteins were 6.21 per cent. At 15 weeks the ulcers were entirely healed but the hair was slightly thin, particularly over the tail, and the skin appeared thin and abnormally pink. The weight was 11.7 kilos, plasma proteins had dropped again to 5.16 per cent, slight icterus was evident, and a bilirubin excretion test showed 42 per cent retention 55 minutes after the 5 minute baseline sample compared with a normal of less than 20 per cent retention. The picture suggests vitamin deficiencies inadequately corrected by the vitamin content of commercial casein. Another dog in current plasmapheresis experiments has not shown any sign of skin lesions after 17 weeks while receiving continuously diet H 30 containing liver powder and yeast powder plus 5 cc. vitamin emulsion. A more complete vitamin emulsion is also being tried.

Amino Acid Feeding Experiments.—Tables 4 and 4-*a*. For 20 weeks following the initial protein depletion period this dog produced fairly abundant plasma protein on a nitrogen intake of the ten essential amino acids plus glycine and on 2 occasions plus other non-essential amino acids. The plasma protein production was good despite the probable vitamin deficiency described in the Experimental History. The slight steady loss of weight persisting in the face of a strongly positive nitrogen balance may have been related to the vitamin deficiency. This point has been discussed above.

The removal of 79.9 gm. of plasma protein during the first 5 periods accomplished experimental protein depletion as described under Methods. About 40 gm. of this protein is estimated to originate in body tissue protein present at the beginning of the experiment (protein reserves) calculated as above for Dog 41-187.

Periods 6 to 16 (Tables 4 and 4-*a*) are devoted to testing reduction in the intake of four of the ten essential amino acids in the mixture previously found (11) very effective for plasma formation. During periods 6 and 7 it appeared that 50 per cent reductions in threonine, lysine, tryptophane, and methionine gave markedly less production than originally observed (11) with mixture Va and than was obtained by its repetition in period 8. Half quantities of threonine, lysine, and methionine tried individually in the mixtures of periods 9 to 12 failed to alter the excellent production response of period 8. It began to appear that the reduction in tryptophane had been responsible for the impaired production of periods 6 and 7, but the continued good production of periods 13 to 16 seemed to overrule this possibility. However, the tryptophane mini-

TABLE 4

Plasma Protein Production with Oral Amino Acid Mixtures for 20 Consecutive Weeks

Dog 40-122

Period 7 days	Diet (Amino acid mixtures Vd, etc., are defined in Table 1-a)	Intake N × 6.25 Total for 7 days		Plasma protein removed Total for 7 days	Blood plasma average concentration		R.B.C. hematocrit, average	Weight
		Amino acids	Basal		Total protein	A/G ratio		
		gm.	*gm.*	*gm.*	*per cent*		*per cent*	*kg.*
	Kennel				6.08	0.95	45.8	11.6
1	Fasting	0	0	34.6	4.92	1.01	46.2	10.6
2	Protein-free	0	2	13.0	3.86	0.84	46.1	10.8
3	Liver	0	72	9.6	4.20	0.62	45.6	10.7
4	Liver	0	72	13.4	4.11	0.76	45.8	10.8
5	Protein-free	0	2	9.3	3.88	0.62	46.2	10.6
6	Vd (Va with ½ lysine, threonine, methionine, tryptophane)	115	2	6.9	3.94	0.71	47.1	10.3
7	Vd	115	2	7.0	4.05	0.71	45.4	10.4
8	Va	114	2	13.5	4.09	0.91	42.7	10.5
9	Ve (Va with ½ threonine)	115	2	16.9	4.30	0.83	43.5	10.6
10	Ve-Vf (Ve with ½ lysine)	116	2	19.8	4.15	0.86	43.0	10.5
11	Vf-Vg	115	2	21.2	4.06	0.68	43.6	10.6
12	Vg (Vf with ½ methionine)	117	2	20.7	4.10	0.75	43.9	10.5
13	Vda (Vd with *dl*-leucine)	115	2	15.3	4.00	0.61	42.8	10.3
14	Vda	115	2	15.8	4.07	0.65	44.8	10.1
15	Vda	115	2	21.1	4.12	0.63	45.5	10.1
16	Vda	115	2	17.8	3.98	0.62	48.3	10.0
17	Vda-VIII	118	2	17.8	3.98	0.57	49.5	9.9
18	VIII	119	2	18.0	4.00	0.66	47.2	9.6
19	Vk (Vda with tripled tryptophane)	116	2	12.7	3.98	0.79	48.0	9.4
20	Vm	77	15	17.8	4.04	0.68	47.5	9.4
21	Vm	77	22	16.1	3.80	0.72	47.0	9.2
22	IX	134	32	20.1	4.05	0.91	46.3	9.2
23	IX	134	32	22.8	4.19	1.08	44.7	9.3
24	IX	134	2	24.9	4.17	0.95	45.3	9.3
25	IX	134	2	22.2	4.12	0.98	46.3	9.2

mum is not absolutely established by this experiment at 0.2 gm. per day for an adult dog of 10 kilos under ordinary circumstances. The technique of plasmaphresis over long periods involves the return of a few more red blood cells than are removed if the hematocrit is to be maintained. This excess affords a possible source of amino acids by recapture from the catabolized hemoglobin. On the other hand, the loss to the body of the tryptophane in the

TABLE 4-*a*

Positive Nitrogen Balance with Amino Acid Mixtures for 20 Consecutive Weeks

Dog 40-122

Period 7 days	Diet (Amino acid mixtures, Vd, etc. are defined in Tables 1-a)	Nitrogen balance						
		Intake			Output			Intake minus output
		In diet		In excess R.B.C. injected	In plasma	In feces	In urine	
		Amino acids	Basal					
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
	Kennel							
1	Fasting		0	4.1	5.7	*	11.1	−14.7
2	Protein-free	0	0.4	0.6	2.1	*	8.5	−11.6
3	Liver	0	11.6	4.5	1.5	*	16.7	−4.1
4	Liver	0	11.6	1.3	2.2	*	12.5	−3.8
5	Protein-free	0	0.4	1.5	1.5	0.7	5.7	−6.0
6	Vd (Va with ½ lysine, threonine, methionine, tryptophane)	18.4	0.3	6.1	1.1	1.2	11.5	+11.0
7	Vd	18.4	0.3	−0.5	1.2	0.7	7.8	+8.5
8	Va	18.2	0.3	2.4	2.2	*	10.4	+6.3
9	Ve (Va with ½ threonine)	18.4	0.3	3.2	2.7	*	12.4	+4.8
10	Ve-Vf (Ve with ½ lysine)	18.6	0.3	4.5	3.2	*	15.1	+3.1
11	Vf-Vg	18.5	0.3	3.5	3.4	*	14.8	+2.1
12	Vg (Vf with ½ methionine)	18.7	0.3	2.8	3.3	*	13.3	+3.2
13	Vda (Vd with dl-leucine)	18.4	0.3	5.8	2.5	2.1	14.0	+5.9
14	Vda	18.4	0.3	4.5	2.5	1.1	14.7	+4.9
15	Vda	18.4	0.6	3.8	3.5	1.5	16.1	+1.7
16	Vda	18.4	0.6	7.5	2.9	2.1	18.1	+3.4
17	Vda-VIII	18.9	0.3	2.5	2.9	0.8	11.0	+7.0
18	VIII	19.1	0.3	3.9	2.9	1.6	11.3	+7.5
19	Vk (Vda with tripled tryptophane)	18.5	0.3	5.2	2.1	1.6	11.8	+8.5
20	Vm	12.4	2.5	4.8	2.9	1.6	10.2	+5.0
21	Vm	12.4	3.6	1.8	2.6	2.0	10.2	+3.0
22	IX	21.5	5.2	3.7	3.3	3.0	11.8	+12.3
23	IX	21.5	5.2	0.8	3.7	2.2	14.0	+7.6
24	IX	21.5	0.3	4.2	4.0	1.2	15.3	+5.5
25	IX	21.5	0.3	5.5	3.6	0.9	12.1	+10.7
	Totals	370.1	45.9	88.0	69.5	42.3	310.4	+81.8

* Fecal nitrogen of 2.0 gm. is estimated for these periods.

plasma protein produced and removed approximately equals what might have been obtained in excess red cells, so that 0.2 gm. per day might be regarded as adequate were it not for the steady rise in urinary nitrogen during periods 13 to 16, abruptly checked by tripling the tryptophane in periods 17 to 19. The minimum probably lies between 0.2 and 0.4 gm. per day for a 10 kilo dog. The data of Rose on the requirement for growing rats (21, 22), that is, tryptophane 0.2 gm. per 100 gm. diet, suggest that a 10 kilo dog might require 0.3 gm. per day.

In periods 17 and 18 added cystine and tyrosine and tripled tryptophane did not make Vda more effective in protein production nor did it alter the slow progression of the skin lesions.

In period 19 (Tables 4 and 4-*a*) plasma protein production appeared to fall but this may have been more apparent than real. If real we have no basis for concluding which of the increases (threonine, phenylalanine, methionine, or arginine) in periods 20 and 21 is most responsible for the prompt renewal of good production. These periods, 20 and 21, demonstrate the uselessness of the excess glycine in the preceding periods.

Periods 22 to 25 show good protein production and positive nitrogen balance but no weight gain. The increase in plasma protein production with this mixture IX is not so great (30 per cent) as the 50 per cent increase in intake of essential amino acids over that of mixture Vm. It would appear that the addition of non-essential amino acids was of no benefit in this experiment; however, other trials of these and other non-essential amino acids might be indicated.

Experimental History—Tables 4 and 4-*a*.

Dog 40-122, an adult female beagle hound was returned to kennel ration after a 64-week experiment reported elsewhere (11). During 7 weeks there was a weight gain from 8.0 kilos to 11.6 kilos and the hair and skin appeared in excellent condition when fasting and plasmapheresis were started in period 1.

In period 2 the proteinless cake, 200 gm., and one unit of vitamin capsules were given. Voluntary food consumption was complete.

In periods 3 and 4 raw pork liver, 50 gm., was added to the daily diet, but it was omitted in period 5. By the end of period 5 erythematous areas, such as preceded the ulcerative skin lesions noted previously in this dog (11) and such as described in Experimental History, Dog 41-187, began to appear over the wrist joints and the chest.

In period 6 amino acids Vd and nicotinic acid, 100 mg., were added to the proteinless cake (150 gm.) and the vitamin capsules.

During periods 7 to 14 skin lesions progressed considerably but food consumption was complete and the dog was otherwise in good clinical condition. In periods 10 and 11 amino acids Ve were given for the first 3 days, Vf for the next 7 days, and Vg for the last 4 days. These skin lesions consisted specifically of a small maculopapular rash over the anterior thorax, the abdomen, and the inner surfaces of the forelegs and thighs plus superficial ulcers over the sternum, the right hip, and the tail plus much general hair loss with conspicuous local loss over the tail and the outer aspects of the legs. The skin was dry, scaly, and over the wrist and ankle joints where ulcers had been previously (11) was purplish-red.

During periods 15 to 25 the dog continued in good condition except for the persistent skin lesions. In periods 15 and 16 the vitamin intake was increased fivefold except for the rice polish concentrate which was raised to 2.5 times the previous level. No improvement in the lesions was detected so that the original quota was resumed in period 18. In period 20 yeast powder, 4 gm., was added to the diet, again without improvement. Liver powder, 4 gm., was started during period 21 and the vitamin emulsion, 10 cc., replaced the vitamin capsules. By the end of period 23 the lesions appeared questionably improved. In periods 24 and 25 the liver and yeast powders were omitted and the lesions appeared as marked as ever.

After period 25 plasmapheresis was continued for 4 weeks. During the first 2 weeks dried foil bakers' yeast (Standard Brands, Inc.) 40 gm., was given with the proteinless cake without added vitamins. Not only were the lesions unimproved but the dog dropped in weight from 9.2 to 8.6 kilos and virtually stopped producing plasma protein. During the 2nd week only 6.0 gm. plasma protein was removed while the average blood plasma protein level dropped to 3.64 per cent. Evidently dried foil yeast is a poor protein source for the dog. In the following 2 weeks plasma protein production was abundant (24.0 and 26.0 gm. respectively) with 30 gm. casein, 20 gm. liver powder, 10 cc. vitamin emulsion, and 50 mg. inositol replacing the yeast. The lesions healed very slowly with the continuance of this diet and the later addition of raw pork liver. Ten weeks after period 25 they were much improved but not entirely gone, a much slower recovery than was obtained on the kennel ration of hospital table scraps after the earlier experimental period.

DISCUSSION

There is much current interest in the value of *parenteral nitrogen administration.* Nitrogen losses following tissue injury from physical trauma, burns, infection, and major surgical procedures may be very large and inadequately covered by oral intakes. Many patients are now forced to repair their injuries by drawing largely upon pre-existing reserves of body protein because of a deficient oral intake of protein. Those with low reserves show hypoproteinemia and heal poorly (10, 26, 27, 8, 20, 19). Those with good reserves may heal their wounds by drawing upon these reserves for some time before clinical evidence of hypoproteinemia appears in the form of an abnormally low plasma protein concentration. Whether or not healing under such circumstances is less rapid and satisfactory than during adequate exogenous nitrogen intake has not been conclusively demonstrated. In any event it appears less desirable because depletion of body protein reserves certainly enhances susceptibility to intercurrent adverse circumstances such as toxins (7, 18, 16, 17) and infection (15, 4, 5, 23). Moreover, since hypoproteinemia is definitely associated with poor wound healing, one should not choose to rely upon a subclinical grade of body protein depletion for repair of injury.

Search for a means of providing protein parenterally when the oral route fails has produced protein digests of definite value (6, 24, 13). Our first trial of solutions of the ten essential amino acids plus glycine suggested that these amino acid solutions were superior to any digests we had tested (11). The data of this report confirm and extend the original observation.

Appropriate mixtures of the ten essential amino acids and glycine when given parenterally are shown to be extremely well tolerated and to provide the nitrogen necessary for good plasma protein production as well as positive nitrogen balance over periods at least as long as several months. The formula for the perfect mixture of amino acids is probably far from completed, but reasonably good mixtures have been found. Many more and long-terms experiments are needed, but certain preliminary, tentative inferences regarding minimum requirements may be drawn.

The Vt mixture has good efficiency in plasma protein production and it promotes a strongly positive nitrogen balance. It is soluble in more than 10 per cent concentration and is well accepted upon rapid injection. Calculating the effective amino acids on the assumption that the unnatural isomers of threonine, valine, leucine, isoleucine, and lysine are largely ineffective (22), the Vt mixture provides, in grams of effective amino acids, threonine 0.35, valine 0.75, leucine 1.5, isoleucine 0.7, lysine 0.6, tryptophane 0.4, phenylalanine 1.0, methionine 0.6, histidine 0.41, arginine 0.4. These quantities appear subminimal in most instances if one translates them into terms of human requirement by multiplying by 4. Such a factor may be adopted on the basis of the estimated relative caloric requirements—750 calories per day for a 10 kilo dog as compared with 3000 for a man (3). In spite of the good nitrogen retention and plasma production, we believe that this mixture was slightly subminimal for the dog in which it was tested. A 100 per cent increase was more than was needed (periods 18 and 19, Tables 3 and 3-*a*), but some increase in all the amino acids except tryptophane and possibly histidine may be warranted. From the data of Tables 4 and 4-*a* the tryptophane in Vt appears more than ample for a minimum mixture. Even *dl*-tryptophane, 0.3 gm daily, was proving fairly effective in the last periods of Tables 3 and 3-*a*.

The use of mixtures of individual amino acids has certain fairly obvious and some not so obvious advantages over the use of protein digests. There is greater and known purity of composition. The composition may be varied if indicated by further work with individuals of different ages and with different disease states. Non-essential amino acids which may be responsible for some of the intolerance to moderately rapid injection of protein digests may be omitted. *Glutamic acid* is such an offender as indicated by vomiting induced by its addition to mixtures of the ten essential amino acids and glycine. This work will be reported. The synthetic solutions of amino acids are better tolerated, may be injected more rapidly, and may be given in high concentration subcutaneously. The leading current disadvantage of amino acid mixtures is their lack of general availability. Large scale production might eliminate this disadvantage and provide amino acids for experimental and clinical use at a cost not out of proportion to their advantages. In the mixtures used the presence of unnatural isomers of the synthetically prepared amino acids shows no sign of toxicity even when given over long periods in the experiments reported here, as well as in unpublished observations in man with administration of amino acids for 75 days. These observations do not support the suggestion of Albanese and Irby (1) that unnatural isomers are toxic.

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 to certain intoxications.

The ten growth essential amino acids of Rose plus glycine will maintain nitrogen balance and produce as much new plasma protein as will good diet proteins. This good utilization is demonstrated over periods of several months when the amino acids are given either orally or parenterally. There is no evidence of toxicity in general nor to unnatural forms of these synthetic amino acids in particular.

Given parenterally appropriate mixtures of these amino acids are well tolerated even upon rapid injection. The minimal daily requirements for a 10 kilo dog may be given intravenously in 10 minutes without reaction. Subcutaneously a 10 per cent solution may be given rapidly without reaction.

Among various mixtures tested Vt approximates a minimum for a 10 kilo dog. It contains in grams *dl*-threonine 0.7, *dl*-valine 1.5, *l*-(−) leucine 1.5, *dl*-isoleucine 1.4, *dl*-lysine hydrochloride 1.5, *l*(−) tryptophane 0.4, *dl*-phenylalanine 1.0, *dl*-methionine 0.6, *l*(+)-histidine hydrochloride 0.5, *l*(+)-arginine hydrochloride 0.5, and glycine 1.0. The presence of glycine improves tolerance to rapid intravenous injection, but excess glycine does not improve utilization of the mixture. Over a long period this mixture appears suboptimal in quantity. Doubled it is more than ample.

Of two casein digests tested the one prepared by enzymatic hydrolysis provided good nitrogen retention and fairly good plasma protein production but was much less tolerable upon intravenous injection than certain mixtures of pure amino acids. The other one prepared by acid hydrolysis and tryptophane fortification afforded bare nitrogen equilibrium and produced virtually no plasma protein.

Skin lesions observed after 10 to 20 weeks of synthetic diet probably reflect

a deficiency of some member or members of the vitamin B_2 group. A persistent slight weight loss in the face of a strongly positive nitrogen balance may accompany this deficiency.

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