ANEMIA AND HYPOPROTEINEMIA

WEIGHT MAINTENANCE EFFECTED BY FOOD PROTEINS BUT NOT BY MIXTURES OF PURE AMINO ACIDS

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It is crystal clear from the experiments tabulated below, in the accompanying Paper I, and elsewhere reported (3, 4) that the doubly depleted dog (anemic and hypoproteinemic) can use the growth mixture of ten essential amino acids to form large amounts of new blood proteins (hemoglobin and plasma protein), to maintain a positive balance between nitrogen intake and urinary nitrogen output, and to continue a healthy state for 3 to 6 weeks. Weight loss, regardless of the amounts of amino acids given by mouth or parenterally, is practically always present and at times approaches or even exceeds the weight loss observed with zero protein intake.

When the amino acid mixture is replaced by comparable amounts of good diet protein (estimated as nitrogen equivalents) we note an improved urinary nitrogen conservation, usually a decrease in new blood proteins produced but a tendency to gain weight on a protein intake of 150 to 250 gm. protein per week. Given a suitable intake of a good food protein plus the basal protein-free diet, the dog will remain in a satisfactory state of double depletion for months with no weight loss and a liberal output of new blood proteins. Evidently the whole protein (egg, liver, meat, casein, lactalbumin) may contain something not found in the amino acid mixtures, as all other factors appear to be alike in the experiments. This missing substance or compound we assume is responsible for the increased conservation of nitrogen and weight gain. It appears probable that this type of experiment (double depletion) with anemia and hypoproteinemia places a severe strain on the animal—more than simple weight maintenance and of a different order than growth requirements. This severe test of double depletion may magnify the deficiencies of any amino acid mixtures or digests when compared with the best food proteins. The pattern of blood protein production in certain experiments is modified by these same factors (amino acids contrasting with food protein). Whatever this unknown substance may be, it is important to learn more about it. Eventually it should be of great value in the construction of amino acid mixtures and digests suitable for long continued optimum parenteral feeding.

Other investigators using different animals and techniques to test growth and maintenance requirements have concluded that something is missing from digests and amino acid mixtures which is present in certain food proteins. Woolley (6, 7) suggests that a factor (strepogenin) may be involved in the nutrition of the mouse. Albanese and Irby (1) using rats showed that mixtures of essential amino acids were inferior from a nutritional point of view to enzymatic or acid hydrolysates of casein or whole casein fed at comparable levels. They suggested that this was due in part to the toxic effects of the unnatural forms of certain amino acids.

Womack and Rose (8) and Rose and Rice (5) state that their experiments with rats point to the presence of an unidentified substance in proteins which is required for maximum increase in weight—a substance not present in amino acid mixtures and certain protein digests Whether one factor like "strepogenin" will acount for all these varied reactions remains to be seen.

Cannon, Wissler, Steffee, Straube, and Frazier (2) in a brief abstract state that a mixture of sixteen pure amino acids patterned after the composition of casein was fed to protein-depleted rats. The rats gained weight almost as fast on the amino acid mixture as on a diet of casein in comparable amounts. When one of the nine essential amino acids was deleted from the mixture there was weight loss and lack of appetite. Return of the deleted amino acid to the mixture was followed by improved food consumption and rapid gain in weight.

EXPERIMENTAL

Table 1 presents interesting comparisons between good food proteins (whole egg and liver) and the growth mixture of amino acids with and without glycine. As might be anticipated no significant differences developed between amino acid mixtures with and without glycine—this in effect is a good control experiment to show the general reaction to the growth mixture of amino acids in varying dosage given parenterally during a 7 week period. Double amounts of the amino acid mixture do not improve the blood protein output nor nitrogen balance (periods 11 and 12). Weight loss is continuous and actually tends to increase in the final periods when the amino acid intake is doubled.

In contrast to the amino acid periods with steady weight loss stand egg protein periods 4 to 5 with a weight gain. There is excellent nitrogen conservation—71.8 gm. intake and 30.2 gm. output in urine. The protein intake in periods 4 to 6 is 222 to 227 gm. and amino acid protein equivalent of periods 11 and 12 is 218 gm. When liver is fed in comparable amounts in periods 13 and 14 (Table 1), we note a prompt reversal of weight loss to weight gain, excellent nitrogen conservation, and blood protein production.

A very similar experiment with *lactalbumen* compared to amino acid mixtures has been recently published (4) (Table 3). Weight loss amounted to 8 per cent in 2 weeks on a high intake of amino acids—339 and 268 gm. respectively each week in protein equivalents. The blood protein output was high—122 gm. per 2 weeks. Lactalbumin, 233 to 248 gm., protein intake in 4 weeks caused a *weight gain* of 1 kilo or 5 per cent. There was zero blood protein production.

Similar responses are noted in Table 2 of Paper I where whole *egg protein* only

TABLE 1

Amino Acid Mixture (Vuj) Minus Glycine and in Doubled Doses
Blood Protein Output—Basal Diet in All Periods
Weight Maintained by Egg and Liver—Not by Amino Acids

Period	Weight	Protein intake		Food consumption	rotein output				Production ratio net protein output to intake	Total nitrogen weekly	
		Type	Weekly		Hemoglobin		Plasma protein			In-take	Urinary output
					Level	Output per wk.	Level	Output per wk.			
1 wk.	*kg.*		*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*gm.*	*gm.*
		Dog 41–52									
1	18.1	Egg	174	86	6.0	1.3	5.2	0		27.8	12.3
2	18.3	Egg	184	91	5.3	25.0	5.3	17.3		29.5	13.6
3	17.7	Egg	132	64	5.3	1.1	5.3	0		21.0	9.9
	0.3 = 2 per cent body weight loss			Totals . . .	(22)	27.4	(24)	17.3	9	78.3	35.8
4	18.0	Egg, yeast digest	227	94	6.1	14.5	5.9	10.7		36.3	16.3
5	18.5	Egg, yeast digest	222	92	5.7	1.2	6.1	0		35.5	13.9
	0.8 = 5 per cent body weight gain			Totals. . .	(27)	15.7	(23)	10.7	11	71.8	30.2
6	17.6	Amino a. − glycine	79	35	7.4	26.8	5.9	20.1		12.1	10.2
7	16.7	Amino a. − glycine	146	37	5.9	27.0	5.5	18.4		22.5	12.4
8	16.5	Amino a. − glycine	107	68	5.9	2.0	4.7	0		16.5	8.4
	2.0 = 11 per cent body weight loss			Totals . . .	(53)	55.8	(22)	38.5	23	51.1	31.0
9	16.5	Amino a. + glycine	109	63	8.9	14.3	4.3	6.5		16.8	9.3
10	15.7	Amino a. + glycine	109	58	8.7	16.6	5.2	7.3		16.8	11.2
	0.8 = 5 per cent body weight loss			Totals . . .	(60)	30.9	(12)	13.8	33	33.6	20.5
11	15.9	Amino a. × 2	218	67	6.5	46.8	4.9	24.3		33.6	21.8
12	14.5	Amino a. × 2	218	29	6.5	1.4	4.6	0		33.6	21.0
	1.2 = 8 per cent body weight loss			Totals . . .	(18)	48.2	(19)	24.3	8	67.2	42.8
13	15.1	Liver	213	100	7.9	24.8	5.1	14.3		34.0	8.0
14	15.3	Liver	213	100	6.8	28.6	4.8	15.5		34.0	9.7
	0.8 = 6 per cent body weight gain			Totals. . .	(57)	53.4	(29)	29.8		68.0	17.7

In all the tables figures in parentheses represent net values corrected for blood volume —see Method.

144 gm. per week caused a 5 per cent gain in 2 weeks. Amino acids 108 gm. per week caused a 4 per cent body weight loss in 3 weeks. There was no significant difference in blood protein production in these two periods. The nitrogen conservation was much greater with the egg protein.

Experimental History—Table 1.

Dog 41-52. Male bull adult, born 1940. Regular double depletion experiments with interspersed recovery periods August, 1945—see Paper I following Table 7.

Mar. 8, 1946—Daily diet of protein-free basal biscuit 450 gm., canned carrots 150 gm., yeast 5 gm., liver extract powder 2 gm., synthetic vitamin mixture 10 cc., choline chloride 600 mg. Blood protein depletion begun. Beginning weight 22.6 kilos, blood volume 1915 cc., plasma volume 991 cc., plasma protein 5.7 gm. per cent. A/G ratio 1.1. Regular double depletion experiments to Apr. 26, 1946.

Apr. 26—Amino acid mixture Vuj *minus glycine,* oral and subcutaneous (Table 1).

Periods 1 to 4. Daily diet of coagulated egg 200 gm., protein-free basal biscuit 350 gm., yeast 3 gm., liver extract powder 2 gm. Plasma volumes 1046 cc., 1044 cc., 1065 cc. A/G ratios 0.98, 1.2, 1.3. Periods 4 and 5. Daily diet of coagulated egg 200 gm., yeast digest (Basamin-Bush) 10 gm., protein-free basal biscuit 300 gm., yeast 3 gm., liver extract powder 2 gm., iron 600 mg., choline chloride 600 mg. Period 4. Protein content per week for egg is 172 gm., for yeast digest 45 gm., for vitamins 10 gm. Period 5. Protein content per week for egg is 168 gm., for yeast digest 44 gm., for vitamins 10 gm. Plasma volumes 1015 cc., 1119 cc. A/G ratios 0.91, 0.89. Period 6. Amino acids incorporated into basal biscuit. Daily diet of amino acid biscuit 350 gm., synthetic vitamin mixture 8 cc., choline chloride 300 mg. Period 7. Amino acid feeding supplemented by amino acid mixture given subcutaneously daily because of poor food consumption. Daily diet of sugar mixture 100 gm., amino acid biscuit 275 gm., synthetic vitamin mixture 10 cc., iron 600 mg. Period 8. Daily diet of protein-free basal biscuit 300 gm., synthetic vitamin mixture 10 cc., iron 1200 mg., choline chloride 600 mg., amino acid mixture subcutaneously. Periods 6 to 9. Plasma volumes 989 cc., 982 cc., 971 cc. A/G ratios 0.71, 0.75, 0.94. Periods 9 and 10. Glycine is incorporated into the amino acid mixture. Total dose is given subcutaneously. Daily diet of protein-free basal biscuit 300 gm., synthetic vitamin mixture 10 cc., iron 1200 mg. Plasma volumes 844 cc., 828 cc. A/G ratios 0.85, 0.90. Periods 11 and 12. Complete Vuj mixture, two standard doses daily (total 40.4 gm.) subcutaneously or vein. Daily diet as of periods 9 and 10. Plasma volumes 848 cc., 853 cc. A/G ratios 1.3, 1.5. Periods 13 and 14. Daily diet of liver 90 gm., protein-free basal biscuit 275 gm., yeast 3 gm. Plasma volumes 839 cc., 805 cc. A/G ratios 1.1 and 1.1. Aug. 2—Daily diet of kennel food and recovery period. Dog in good condition other than weight loss.

Table 2 presents data on *casein* as compared with varying doses of an amino acid mixture. The experiment is quite satisfactory and the consumption of the diet mixtures adequate. The amino acid mixtures were all given subcutaneously. *Casein* in amounts of 204 gm. protein per week maintains an even *weight balance* and good average blood protein output—the ratio of protein output to intake is 20 per cent.

Amino acids in approximately the same amount for 1 week (period 6) show a 5 per cent body *weight loss* and a positive nitrogen balance. The blood protein output decreases.

Amino acids (periods 7 and 8) in about one-half the standard dose for 2 weeks show a continuing *weight loss* (8 per cent), a slight positive nitrogen balance, and an in-

TABLE 2

Amino Acid Mixture Vaa in Varying Dosage—Subcutaneous

Casein in contrast—basal diet in all periods.

Period	Weight	Protein intake: Type	Protein intake: Weekly	Food Consumption	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Output per wk.	Protein output: Plasma protein: Level	Protein output: Plasma protein: Output per wk.	Production ratio net protein output to intake	Total nitrogen weekly: Intake	Total nitrogen weekly: Urinary output
1 wk.	*kg.*		*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*gm.*	*gm.*
		Dog 40-43									
1	13.9	Basal	19	100	10.0	118.2	4.6	48.9			
2	13.3	Basal	19	100	9.3	19.4	4.6	6.8			
3	12.6	Casein	204	100	9.3	28.4	4.6	13.0			
4	13.2	Casein	204	100	7.6	38.7	4.9	17.7			
			Totals........		(54)	67.1	(26)	30.7	20		
5	13.0	Amino a. × 2	134	84	9.3	14.2	4.5	6.9		21.2	19.0
6	12.4	Amino a. × 2	202	74	9.3	1.8	4.0	0		32.3	22.9
		0.8 = 6 per cent body weight loss..................	Totals.......		(32)	16.0	(0)	6.9	9	53.5	41.9
7	12.0	Amino a. ½ standard dose	50	76	6.9	39.7	4.9	19.1		8	7.3
8	11.4	Amino a. ½ standard dose	50	81	7.7	9.8	4.6	6.2		8	6.2
		1.0 = 8 per cent body weight loss	Totals.......		(32)	49.5	(29)	25.3	61	16	13.5
9	11.5	Amino a. standard dose	100	78	7.4	25.2	4.7	12.0		16	8.9

TABLE B

Amino Acid (Vaa) Growth Mixture Used (Table 2)

	gm.		*gm.*
dl-Threonine................	1.4	*dl*-Phenylalanine...........	2.0
dl-Valine....................	3.0	*dl*-Methionine..............	1.2
dl-Leucine..................	4.4	*l* (+) Histidine HCl.........	1.0
dl-Isoleucine................	2.0	*l* (+) Arginine HCl.........	1.0
l (+) Lysine HCl...........	2.2	Glycine....................	2.0
dl-Tryptophane.............	0.6		
		Total amino acid...........	20.8

Protein equivalent = 19.74 gm., nitrogen = 2.75 gm. for total daily dose. Unnatural isomeric forms = 28 per cent of total nitrogen.

creased output of blood proteins. This experiment again emphasizes the striking difference between a standard food protein casein and amino acid mixtures relating to weight maintenance or weight loss.

Experimental History—Table 2.

Dog 40-43. Male bull. Born 1940. Maintained on regular kennel diet. Nov. 18, 1942—Daily diet of protein-free basal biscuit 400 gm., cod liver oil 10 cc., yeast 5 gm., liver extract powder 5 gm., iron 400 mg. Blood protein depletion begun. Beginning blood volume 1070 cc., plasma volume 545 cc., plasma protein 6 gm. per cent. Regular double depletion experiments with interspersed recovery period. Mar. 30, 1943—Daily diet of protein-free basal biscuit 400 gm., yeast 3 gm., liver extract powder 2 gm., iron 600 mg. Blood protein depletion begun. Plasma volume 678 cc., weight 14.9 kilos, plasma protein 5.8 gm. per cent.

Apr. 7—Amino acid mixture Vaa in varying dosage, subcutaneous (Table 2).

Periods 1 and 2. Daily diet as of Mar. 30 except that synthetic vitamin mixture replaces yeast and liver extract powder. Plasma volumes 761 cc., 689 cc. Period 2. A/G ratio 1.0. Periods 3 and 4. Daily diet of casein 30 gm., protein-free basal biscuit 400 gm., yeast 3 gm., liver extract powder 2 gm., iron 600 mg. Plasma volumes 635 cc., 704 cc. A/G ratios 1.1, 0.95. Periods 5 and 6. Amino acid mixture Vaa two standard doses daily (total 41.6 gm.) subcutaneously. Daily diet of protein-free basal biscuit 400 gm., synthetic vitamin mixture 8 cc., choline chloride 200 mg., iron 600 mg. Plasma volumes 710 cc., 689 cc. A/G ratios 1.2, 1.4 Periods 7 and 8. Amino acid mixture Vaa ½ standard dose daily (total 10.4 gm.). Daily diet of protein-free basal biscuit 300 gm., synthetic vitamin mixture 8 cc., choline chloride 100 mg., iron 600 mg. Plasma volumes 629 cc., 691 cc. A/G ratios 1.2, 1.1. Period 9. Amino acid mixture one standard dose daily (total 20.8 gm.) subcutaneously. Diet as of periods 7 and 8. Plasma volume 874 cc. A/G ratio 1.4. May 27—Kennel diet and recovery period. Dog in good condition other than weight loss.

Table 3 gives some information on *three amino acids* (methionine, threonine, and phenylalanine) which effected such definite nitrogen conservation in the preceding paper I. When each of these amino acids was deleted from the growth mixture of amino acids there was a definite rise in urinary nitrogen which returned to normal when the individual amino acid was returned to the standard amino acid mixture (see Tables 1 to 3, Paper I). We thought that the three amino acids (methionine, threonine, and phenylalanine) given together under these experimental conditions might yield an interesting response. Leucine was added in one experiment and lysine in the second experiment. Table 3 shows that the effect is largely negative, the nitrogen balance is negative although one must admit that some nitrogen is retained (dog 43–347). The basal protein-free diet shows about 6 gm. urinary nitrogen excreted a week—if these animo acids were all excreted the weekly total urinary nitrogen would be about 16.6 gm.—actually about 13 gm. nitrogen is found or a conservation of 3.6 gm. The blood protein output is no greater than during basal periods alone (raiding of body protein). Period 1 (dog 43-347), Table 3, shows the removal of a large reserve store of protein-building material in the early stage of the depletion.

Liberal diet protein (salmon bread and mixed proteins) reverses the weight loss of 12 per cent to a weight gain of 7 per cent. The blood protein output of 25 gm. per week associated with the amino acid intake increased to about 40 gm. blood protein per week due to the mixed diet. Methionine alone (Table 3) was added to the

TABLE 3

Methionine, Threonine, Phenylalanine, and Leucine Given Parenterally

Period	Weight	Protein intake: Type	Protein intake: Weekly	Food consumption	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Output per wk.	Protein output: Plasma protein: Level	Protein output: Plasma protein: Output per wk.	Production ratio net protein output to intake	Total nitrogen weekly: Intake	Total nitrogen weekly: Urinary output
1 wk.	*kg.*		*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*gm.*	*gm.*
		Dog 43–347									
1	15.8	Basal	19	100	8.7	105.1	5.7	38.5			
2	14.4	Basal	17	94	8.3	36.6	5.0	22.0			
3	13.7	Basal	15	80	6.6	24.6	4.5	12.1			
4	13.0	Amino a., all four	66	57	7.5	13.9	4.4	6.7		9.6	12.0
5	12.6	Amino a., all four	73	45	7.9	13.7	4.4	6.2		10.8	15.9
6	12.1	Amino a., all four	73	32	8.2	18.9	4.2	9.8		10.8	11.0
	1.6 = 12 per cent body weight loss	Totals.....			(58)	46.5	(18)	22.7	30	31.2	38.9
7	12.1	Salmon bread	248	86	9.0	15.3	5.0	7.3		39.6	18.8
8	12.4	Salmon br. + kennel	472	96	9.2	26.7	4.6	11.9		75.4	17.9
9	12.6	Salmon br. + kennel	482	95	9.0	35.4	4.9	18.5		77.1	
10	12.9	Salmon br. + kennel	538	98	7.2	31.7	4.6	16.7		86.0	
	0.8 = 7 per cent body weight gain	Totals....			(103)	109.1	(60)	54.4	9		
11	12.3	Basal	14	72	6.9	12.8	4.7	7.7		2.2	6.2
12	11.3	Methionine 1.5	6.3	42	6.9	13.9	4.5	7.0		1.0	5.6
13	11.1	Methionine 1.5	6.3	39	7.2	1.5	4.5	0		1.0	6.2
14	10.6	Methionine 1.5	6.3	48	8.1	1.7	4.2	—		1.0	5.3
	1.7 = 14 per cent body weight loss	Totals....			(24)	17.1	(−1)	7.0			

Methionine, threonine, phenylalanine, and lysine

Period	Weight	Protein intake: Type	Protein intake: Weekly	Food consumption	Protein output: Hemoglobin: Level	Protein output: Hemoglobin: Output per wk.	Protein output: Plasma protein: Level	Protein output: Plasma protein: Output per wk.	Production ratio net protein output to intake	Total nitrogen weekly: Intake	Total nitrogen weekly: Urinary output
		Dog 43–174									
1	23.6	Basal	19	100	11.2	66.8	5.2	25.0		3.0	
2	22.8	Basal + carrots	29	100	10.4	39.3	4.2	14.4		4.6	
3	22.0	Amino a. × 2, all 4	94	59	10.0	17.0	4.2	6.1		15	16.7
4	21.7	Amino a. × 2, all 4	94	59	10.0	2.2	4.2	1.0		15	11.6
5	21.7	Amino a. × 2, all 4	94	64	11.0	2.2	4.3	1.0		15	14.4
	1.1 = 5 per cent body weight loss	Totals...			(20)	21.4	(4)	8.1		45	42.7
6	20.7	Liver	100	100	12.9	31.2	4.3	10.1		16.2	12.3
7	20.5	Liver	92	92	12.0	2.6	4.1	1.0		15.0	8.2
8	19.6	Liver	92	92	12.1	20.0	4.2	5.0		15.0	9.1
	2.0 = 10 per cent body weight loss	Totals....			(56)	53.8	(19)	16.1			
9	19	Liver	202	100	12.9	2.7	4.7	0		32.0	9.6

basal diet in periods 12 to 14 with negative effect as far as nitrogen conservation is concerned. Blood protein output falls.

A second experiment (Table 3) with methionine, threonine, phenylalanine, and lysine in slightly larger amounts shows little if any difference between dog 43–174 and dog 43–347 (Table 3).

Experimental History—Table 3.

Dog 43-347. Male terrier. Maintained in laboratory kennels for several months under optimum conditions. Apr. 6, 1946—Daily diet of protein-free basal biscuit 400 gm., canned onions 150 gm., yeast 3 gm., liver extract powder 2 gm., choline chloride 300 mg. Blood protein depletion begun. Beginning blood volume 1263 cc., plasma volume 692 cc., plasma protein 6.2 gm. per cent.

Apr. 27—Amino acid mixture subcutaneously six doses per week (Table 3).

Period 4. Daily dose of *dl*-methionine 1.8 gm., *dl*-threonine 3.3 gm., *dl*-phenylalanine 2.1 gm., *l* (−) leucine 4.7 gm., glycine 2 gm. Daily diet of protein-free basal biscuit 300 gm., synthetic vitamin mixture 10 cc., choline chloride 300 mg. Periods 5 and 6. Amino acid mixture daily dose of *dl*-methionine 1.8 gm., *dl*-threonine 3.3 gm., *dl*-phenylalanine 2.1 gm., *l* (−) leucine 4.7 gm., glycine 3 gm. subcutaneously for 6 days per week. Plasma volumes (periods 4 to 7) 682 cc., 717 cc., 626 cc. A/G ratios 1.7, 1.8, 1.8. Period 7. Daily diet of salmon bread 300 gm., protein-free basal biscuit 50 gm., liver extract powder 2 gm., iron 600 mg. Plasma volume 629 cc. A/G ratio 1.6. Dog appears listless. Choline chloride 200 mg. added to diet. Period 8. Daily diet of salmon bread 325 gm., kennel diet 200 gm., yeast 3 gm., liver extract powder 2 gm., salt mixture 5 gm. Dog slightly more active. Plasma volume 636 cc. A/G ratio 1.6. Periods 9 and 10. Kennel diet increased to 700 gm., salmon bread decreased to 250 gm., iron 600 mg. added. Plasma volumes 644 cc., 689 cc. A/G ratios 1.5, 1.4. Dog much livelier. Period 11. Daily diet of protein-free basal biscuit 350 gm., yeast 3 gm., iron 600 mg. Plasma volume 703 cc. A/G ratio 1.3. Periods 12 to 14 inclusive. *dl*-Methionine 1.5 gm., added to synthetic vitamin mixture and fed. Daily diet of protein-free basal biscuit 350 gm., iron 600 mg. Plasma volumes 646 cc., 614 cc., 597 cc. A/G ratios 1.5, 1.4, 1.0. Experiment terminated, dog in good condition other than weight loss.

Dog 43-174 continued from "Experimental history" Table 8, Paper I.

June 27, 1946—Daily diet of protein-free basal biscuit 400 gm., yeast 3 gm., liver extract powder 2 gm. Blood protein depletion begun. Beginning weight 24.3 kilos, blood volume 1844 cc., plasma volume 1197 cc. A/G ratio 2.1.

July 12—Amino acid mixture subcutaneously six doses per week (Table 3). Periods 3 to 6. *dl*-Methionine 2.4 gm., *dl*-threonine 4.4 gm., *dl*-phenylalanine 2.8 gm., *l* (+) lysine HCl 5.0 gm. Daily diet of protein-free basal biscuit 450 gm., dextrose 20 gm., synthetic vitamin mixture 8 cc., choline chloride 150 mg. Periods 3 to 6. Plasma volumes 1100 cc., 1125 cc., 1058 cc. A/G ratios 1.4, 1.9, 1.5. Periods 6 to 9. Daily diet of liver 45 gm., protein-free basal biscuit 300 gm. Plasma volumes 1020 cc., 1023 cc., 968 cc. A/G ratios 1.1, 1.1, 1.2. Period 9. Daily diet of liver 90 gm., protein-free basal biscuit 300 gm., synthetic vitamin mixture 8 cc., choline chloride 300 mg. Plasma volume 916 cc. A/G ratio 1.6. Aug. 31—Kennel diet and recovery period. Dog in good condition other than weight loss.

SUMMARY

Dogs with sustained anemia and hypoproteinemia due to bleeding and a continuing low protein or protein-free diet with abundant iron are used to test the value of food proteins as contrasted with mixtures of pure amino acids.

The stimulus of double depletion (anemia and hypoproteinemia) drives the body to use every source of protein and all protein-building materials with the utmost conservation. Raiding of body tissue protein to produce plasma protein and hemoglobin is a factor when protein-building factors are supplied in small amounts.

In this severe test (double depletion) the good food proteins in adequate amounts are able to maintain body weight, a strongly positive nitrogen balance, and produce considerable amounts of new hemoglobin and plasma protein. Casein, lactalbumin, whole egg protein, liver protein are all adequate in amounts of 150 to 250 gm. protein per week.

Under comparable conditions mixtures of pure amino acids (essential for growth) do produce large amounts of new hemoglobin and plasma protein and a positive nitrogen balance but do not maintain body weight. The loss of weight is conspicuous even with large amounts of amino acids (200 to 300 gm. protein equivalent per week).

Methionine, threonine, and phenylalanine are related to nitrogen conservation in growth mixtures of essential amino acids (Paper I) but when these three are given together they have little influence on the doubly depleted dog (Table 3).

Some unidentified substance or compound present in certain proteins but absent in mixtures of the essential amino acids may be responsible for these differences in the response of the doubly depleted dog.

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