PLASMA PROTEIN AND HEMOGLOBIN PRODUCTION*

Deletion of Individual Amino Acids from Growth Mixture of Ten Essential Amino Acids. Significant Changes in Urinary Nitrogen

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The evidence given below in table form indicates clearly that certain amino acids have a fundamental influence on the conservation of nitrogen in the dog. When methionine or threonine (Tables 1 and 2) are withdrawn from the amino acid growth mixture there is a sharp rise in urinary nitrogen even to the point of a negative urinary nitrogen balance. There may or may not appear some change in blood protein production and body weight loss. Phenylalanine and tryptophane in similar fashion have a definite influence upon nitrogen retention and tryptophane (Table 4) appears to stimulate the production of plasma proteins when returned to the amino acid mixture. Histidine, valine, and lysine in some experiments show a moderate effect upon nitrogen elimination. Arginine, leucine, and isoleucine are without effect upon the urinary nitrogen in these experiments within the 3 or 4 week periods of observation.

When the deleted individual amino acid is replaced in the amino acid mixture the response may be prompt or sometimes delayed. Likewise when the complete amino acid mixture is followed by a standard food protein period there sometimes is a delayed response suggesting that the body requires some days to adjust to a favorable diet period and resume the expected blood protein output. Long continued depletion periods of inadequate diet or amino acid mixtures surely place a severe burden on the depleted dog—reserves are dangerously low and one may observe a lag in the response at the start of an adequate diet period. There may be less gain in weight or less blood protein output than would be anticipated and one suspects that the nitrogenous materials are going into tissue reserve stores which must be repleted before the increase in blood protein output becomes evident.

It perhaps will surprise the casual reader to note that this double stimulus of anemia and hypoproteinemia will call out new hemoglobin and blood plasma proteins in dogs whose *labile* reserve stores are exhausted. This feature is emphasized in Paper III below and we use the term "raiding of body protein"

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to describe this emergency production of blood proteins—the blood proteins have a priority over the organ and tissue proteins in this type of emergency.

This type of experiment presents many difficulties which may force premature termination of an experiment in mid-flight. These depleted dogs (anemic and hypoproteinemic) become tired of the basal non-protein diet and lack of food intake may compel termination of some experiments. The condition may deteriorate, and rather than take a chance on losing a valuable dog, the experiment is terminated. These incomplete experiments give some information of value and their data are in harmony with the experiments tabulated below. Difficult and in a measure unsatisfactory as they are, these amino acid exclusion experiments do give unexpected information of some interest to workers in this field.

Parenteral nutrition is a broad field and these experiments touch this area. The actual influence of single amino acids upon body nutrition is not known and yet it is not unlikely that there are conditions in which a lack of one amino acid is of profound importance in health or disease. For example methionine aids in nitrogen conservation under certain conditions (5), and in protection of the liver against some poisons (3, 4). Threonine appears to have a profound and prompt influence upon urinary nitrogen retention or elimination (Table 2). *Tryptophane* may stimulate plasma protein production in the depleted dog (Table 4) under certain conditions. With these illustrations in mind it is obvious that further information will be of interest and should be obtained. Supplementation of proteins and concentrates for feeding in abnormal states or fortification of digests by important amino acids or other organic compounds may bring improvements in our crude techniques for parenteral feeding.

These three papers are in reality one contribution and the discussion or bibliography of any one is incomplete without the other two papers.

Methods

The dogs used in these experiments represent two types. Those taken from the anemia colony represent a white bull terrier and coach strain. They are raised in the laboratory kennels. The other dogs are obtained from outside sources. They are maintained under optimum dietary conditions and kept under constant supervision for a period of several months preceding the experiments. Great care is taken to protect these animals from infection by proper housing, isolation, vaccination, and handling. Dogs belonging to the anemia colony are maintained at hemoglobin and red cell levels of about ⅓ their original levels by bleeding and a diet of the standard salmon bread during their anemia period. Subsequent depletion of their plasma proteins to produce *double depletion* is accomplished during a 2 to 3 week period. Blood protein depletion of non-anemic dogs necessitates a 4 to 6 week period. All experiments concern "double depletion" (anemia and hypoproteinemia) produced by blood removal and a low or non-protein diet plus abundant iron. This diet with accompanying weight loss cannot be continued indefinitely; recovery periods are therefore interspersed in the experimental program. General technical procedures concerning the animals and experiments proper have been described previously (6–8).

The *basal non-protein ration* is a biscuit containing adequate carbohydrates, fat, minerals, including ample iron, and choline chloride (6). Animals tiring of this are offered a change in diet in the form of a sugar mixture the components of which are: dextrose 200 gm., cane sugar 600 gm., bone ash 78 gm., salt mixture (McCollum and Simmonds) (8), liver extract powder (Lilly) 26 gm., yeast 26 gm., (Standard Brands, Type 200B), Mazola oil 236 cc., cod liver oil 50 cc., and melted lard 490 gm.

Vitamin additions to the diet consist of dried yeast (Standard Brands, Inc., Type 200B) and a liver powder prepared from pig liver. The amount of nitrogen they contain (N × 6.25) has been added to the protein intake indicated in the tables. In amino acid deletion experiments a synthetic vitamin mixture is used in order to limit the nitrogen intake to the amino acid mixture.

Casein, canned salmon, and canned squash are commercial products. Beef is lean ground round steak. Liver is fresh, cooked pig liver. Desiccated horse liver is a dried product supplied by Arlington Chemical Co. "Basamin-Bush" is a commercial yeast digest. Eggs are beaten, coagulated in a double boiler, and fed shortly after preparation.

Amino acids deleted from the Vuj growth mixture are indicated in tables. For parenteral administration amino acids are dissolved in distilled water, brought to the boiling point, filtered, and transferred to sterile drip bottle. For oral administration amino acids are incorporated into the basal ration biscuit.

TABLE A

Amino Acid (Vuj) Growth Mixture Used in All Experiments (Paper I)

Daily intake

	gm.		*gm.*
dl-Threonine	2.2	*dl*-Phenylalanine	1.4
dl-Valine	2.8	*dl*-Methionine	1.2
l (−) Leucine	3.1	*l* (+) Histidine HCl	0.8
dl-Isoleucine	2.2	*l* (+) Arginine HCl	1.6
l (+) Lysine HCl	2.5	Glycine	2.0
dl-Tryptophane	0.4	*Total* amino acids	20.2

Protein equivalent = 18.15 gm.; nitrogen = 2.8 gm. for total daily dose. Unnatural isomeric forms = 20 per cent of total nitrogen.

For *urinary nitrogen* analyses the dogs are kept in metabolism cages, the urine is collected daily and transferred to a storage bottle containing 200 cc. concentrated sulfuric acid. The 7 days' collection is measured, filtered, and aliquot samples taken for nitrogen determination by macroKjeldahl method.

In the following tables for any given dog weekly periods run consecutively. Hemoglobin and plasma protein outputs are given in the tables in grams of material actually removed by bleeding and measured. Hemoglobin levels are those obtained by sampling 48 hours following blood removal in case of a single bleeding. In case of repeated bleedings during the week the hemoglobin level is obtained during the blood volume determination at the end of the week. Plasma protein levels represent the average of samples of each bleeding during the week. "Output per week" is the total hemoglobin or plasma protein removed during the week. The figures in parentheses are values indicating the "net corrected total output." These figures are the amounts of blood protein actually removed by bleeding plus or minus the calulated amounts related to differences in the circulating levels of hemoglobin and plasma proteins as determined by blood volumes at the start and at the end of the given period.

EXPERIMENTAL OBSERVATIONS

Table 1 (Dog 41-50) presents a clean cut experiment to show how important *methionine* is for *nitrogen conservation*. When methionine is replaced in the amino acid mixture there is a sharp drop in urinary nitrogen, diminished weight loss, and an increase in the output of blood proteins. Period 5 (Table 1) shows an increase in urinary nitrogen of about 6 gm. which is adequately explained by a small subcutaneous abscess in the flank, promptly opened and drained, lasting 3 days. When a natural protein (egg) in like amounts replaces the growth mixture of amino acids there is even more conservation of urinary nitrogen and a little less blood protein production but continued weight loss. Here the complete amino acid growth mixture compares well with an excellent food protein. Periods 6 and 7 show a total urinary nitrogen of 19.6 gm. and periods 8 and 9 show total urinary nitrogen of 11.1 gm. Some of the surplus in periods 6 and 7 is due to unnatural isomers of amino acids (3.0 gm. N) and some to the intravenous injection of amino acids which are less well used than amino acids given by mouth.

There is other evidence of the importance of methionine in internal metabolism. When methionine is replaced by cystine in the growth mixture in experiments dealing with plasma protein production in hypoproteinemia (Table 1) (1), there is loss of weight, negative nitrogen balance, and good plasma protein output for a limited period of time. Methionine has a protein-sparing effect in dogs on a very low protein diet (5) and methionine protects against chloroform poisoning even when given 3 to 4 hours after chloroform anesthesia (3, 4).

TABLE 1

Amino Acid Growth Mixture Minus Methionine—Intravenous Blood Protein Output—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 41-50									
1	19.1	Basal	17	87	7.0	30.9	4.6	16.7		2.7	9.9
2	18.5	Amino a. − methionine	105	86	7.3	8.7	4.3	5.0		16.2	13.5
3	17.9	Amino a. − methionine	105	91	8.0	18.3	4.3	7.1		16.2	18.6
4	17.8	Amino a. − methionine	105	91	7.6	12.3	4.2	6.0		16.2	13.7
5	16.4	Amino a. − methionine	105	66	6.3	25.3	4.5	13.0		16.2	19.8
	2.7 = 14 per cent body weight loss	Totals.......			(43)	64.6	(24)	31.2	16	64.8	65.6
6	16.5	Amino a. + methionine	109	60	6.3	1.6	4.1	0		16.8	10.9
7	15.5	Amino a. + methionine	109	67	6.4	29.2	4.7	16.1		16.8	8.7
	0.9 = 5 per cent body weight loss	Totals......			(31)	30.8	(19)	16.1	23	33.6	19.6
8	15.4	Whole egg	108	100	5.1	12.0	4.2	7.9		16.8	5.8
9	14.8	Whole egg	108	100	6.8	1.8	4.9	0		16.8	5.3
	0.7 = 5 per cent body weight loss	Totals.......			(18)	13.8	(10)	7.9	13	33.6	11.1

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

Experimental History—Table 1.

Dog 41–50. Male coach. Born January, 1940. Continuous anemia history Feb. 16, 1943, to June 22, 1943. Regular anemia experiments. Beginning weight 15.3 kilos. Blood volume 1075 cc., plasma volume 508 cc. June 22, 1943—Daily diet of protein-free basal biscuit 450 gm., yeast 5 gm., reduced iron 600 mg. Plasma protein depletion begun. Regular double depletion experiments with interspersed recovery periods. Aug. 9, 1944—Daily diet of protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Blood protein depletion begun. Plasma volume 948 cc. Weight 22.1 kilos. Plasma protein level 5.7 gm. per cent.

Sept. 7—Amino acid mixture Vuj ***minus dl-methionine*** by vein (Table 1). Daily diet of protein-free basal biscuit 450 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Periods 2 to 6. Plasma volumes 1073 cc., 994 cc., 1015 cc., 886 cc. A/G ratios 1.2, 0.6, 0.9, 0.96. Sept. 28—Small abscess on flank, drained, duration 3 days. Periods 6 and 7. *dl*-Methionine added to amino acid mixture. Daily diet as of Sept. 7. Plasma volumes 986 cc., 850 cc., A/G ratios 1.0, 1.2. Periods 8 and 9. Daily diet of whole egg 115 gm., protein-free basal biscuit 450 gm., synthetic vitamin mixture 10 cc., reduced iron 600 mg. Plasma volumes 920 cc., 925 cc. A/G ratios 0.9, 0.86. Plasma indicates hemolysis. No *Bartonella* in blood smears. Nov. 1—Experiment terminated. Skin lesions. Dog in poor condition. Daily diet of liver and salmon bread. Rest and recovery period.

Table 2 (Dog 37-85) presents a convincing experiment to indicate the importance of *threonine* for *nitrogen conservation*. When threonine is omitted from the essential growth mixture of amino acids, the uruinary nitrogen balance is negative and there is rapid loss of weight. When threonine is replaced in the mixture, the urinary nitrogen balance becomes positive and the weight loss is only one-third that of the preceding 3 week period. Food consumption was excellent in all periods. It is of interest to note that the net output of blood proteins is greater in the absence of threonine, 96 gm. total blood protein output to 37 gm. blood protein for an equal length of time when threonine is replaced. "Raiding of proteins" in the body (weight loss) to form the needed blood proteins probably is a part of this reaction—see Paper III. When egg (144 gm. protein per week) is fed in place of the amino acid mixture with or without threonine, we note a sharp change to weight gain and greater urinary nitrogen conservation—a contrast of 5 per cent weight gain with a 4 per cent weight loss in periods 5 to 7 (Table 2). The amino acids were given in somewhat smaller protein equivalents (108 gm. protein).

Further evidence of the nitrogen conservation related to threonine is found in another paper from this laboratory (Table 2 (1)). When *threonine* was omitted from the growth mixture of essential amino acids there was a sharp rise in urinary nitrogen and fall in plasma protein production in the hypoproteinemic dog.

Experimental History—Tables 2 and 5.

Dog 37–85. Male coach. Born August, 1936. Continuous anemia history Nov. 13, 1940, to Aug. 4, 1943. Regular anemia experiments. Beginning weight 17.4 kilos, blood volume 1540 cc., plasma volume 791 cc. Aug. 4, 1943—Daily diet of protein-free basal biscuit 400 gm., yeast 5 gm., reduced iron 600 mg. Plasma protein depletion begun. Regular double depletion experiments with interspersed recovery periods. Mar. 30, 1944—Daily diet protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Blood protein depletion begun. Plasma volume 954 cc., weight 21.7 kilos. Apr. 27—Daily diet protein-free basal biscuit 450 gm., casein 15 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Plasma volume 976 cc. A/G ratio 1.1.

May 4, 1944—Amino acid mixture Vuj *minus l* (−) *leucine* by mouth (Table 5). Amino acids incorporated into basal biscuit. Daily diet of amino acid biscuit 210 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Periods 2 to 5. Plasma volumes 1028 cc., 887 cc., 833 cc. A/G ratios 0.76, 0.79, 0.84. Dog in good condition. Periods 5 and 6. Daily diet of liver 200 gm., lean beef 200 gm., salmon bread 200 gm., yeast 3 gm., liver extract powder

TABLE 2

Amino Acid Growth Mixture Minus Threonine—Subcutaneous Blood Protein Output—Basal Diet in All Periods

Period	Weight	Protein intake		Food consumption	Protein output				Production ratio net protein output to intake	Total nitrogen weekly	
		Type	Weekly		Hemoglobin		Plasma protein			Intake	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 37-85									
1	18.5	Basal	19	100	9.3	35.8	4.3	14.6		3.0	—
2	18.2	Amino a. − threonine	99	100	9.8	28.3	4.6	12.6		15.9	17.2
3	16.7	Amino a. − threonine	99	100	9.5	25.2	4.4	11.3		15.9	16.9
4	16.1	Amino a. − threonine	99	82	8.5	30.5	4.1	14.5		15.9	19.1
	2.4 = 13 per cent body weight loss	Totals......			64	84.0	(32)	38.4	32	47.7	53.2
5	16.1	Amino a. + threonine	108	85	8.1	11.0	4.7	5.5		16.8	11.1
6	15.8	Amino a. + threonine	108	100	6.9	23.7	4.3	13.7		16.8	11.0
7	15.5	Amino a. + threonine	108	100	6.9	1.5	4.3	0		16.8	11.6
	0.6 = 4 per cent body weight loss	Totals.......			(15)	36.2	(22)	19.2	11	50.4	33.7
		Interval of some wks.									
	16.5	Egg	144	100	9.0	12.4	4.5	5.6		23.0	7.2
	17.3	Egg	144	100	8.6	14.4	4.4	6.4		23.0	10.0
	0.8 = 5 per cent body weight gain	Totals......			(27)	26.8	(14)	12.0	14	46.0	17.2

2 gm., reduced iron 600 mg. Plasma volumes 864 cc., 857 cc. Period 6. A/G ratio 0.96 Dog in excellent condition, rapidly gaining weight. June 23—Daily diet of kennel food and recovery period. Sept.7—Daily diet of protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Blood protein depletion begun. Beginning plasma volume 876 cc. Weight 20.2 kilos.

Sept. 22—Amino acid mixture Vuj *minus dl-threonine* subcutaneously (Table 2). Daily diet of preotein-free basal bisuecuit 450 gm., synthetic vitamin mixture 10 cc., reduced iron 600 mg. Periods 2 to 5. Plasma volumes 944 cc., 865 cc., 856 cc. A/G ratios 0.94, 0.86, 0.9. Periods 5 to 7 inclusive. *dl*-Threonine added to amino acid mixture. Daily diet as of Sept. 22. A/G ratios 1.2, 1.0, 0.93. Periods 6 and 7. Plasma volumes 852 cc., 900 cc. Dog in good condition.

Table 3 (Dog 40-34) presents data on a good experiment with and without *phenylalanine.* When phenylalanine is absent from the amino acid growth mixture, the urinary nitrogen is high but when phenhlalanine is replaced in periods 6 and 7, the urinary nitrogen falls and urinary nitrogen balance becomes strongly positive. The loss of weight is the same per week in all periods (4 per cent). With phenylalanine present or absent in the mixture, the corrected blood protein output per week is about the same—48 and 51 gm. Food consumption of the basal diet was satisfactory. When casein is given the nitrogen balance is more favorable but the blood protein output falls.

The weight loss on casein is one-half that of the amino acid in periods 6 and 7 (Table 3).

TABLE 3

Amino Acid Growth Mixture Minus Phenylalanine—Vein and Subcutaneous Blood Protein Output—Basal Diet in All Periods

Period	Weight	Protein intake		Food consumption	Protein output				Production ratio net protein output to intake	Total nitrogen weekly	
		Type	Weekly		Hemoglobin		Plasma protein			Intake	Urinary output
					Level	Output per wk.	Level	Outper per wk.			
1 wk.	*kg.*		*gm.*	*per cent*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*gm.*	*gm.*
		Dog 40-34									
1	18.2	Basal	15	79	10.0	40.8	4.9	24.4		2.4	—
2	17.8	Amino a. − phenylalanine	87	57	11.1	13.1	3.6	3.5		13.9	10.1
3	17.2	Amino a. − phenylalanine	104	70	9.1	35.4	5.0	16.0		16.6	19.0
4	16.3	Amino a. − phenylalanine	104	75	7.8	24.2	4.6	13.2		16.6	14.8
5	15.5	Amino a. − phenylalanine	104	69	7.8	1.7	4.3	0		16.6	17.8
	2.7 = 15 per cent body weight loss		Totals......		(26)	74.4	(22)	32.7	12	63.7	61.7
6	14.7	Amino a. + phenylalanine	113	71	7.4	21.3	4.5	12.7		17.6	13.7
7	14.2	Amino a. + phenylalanine	113	79	6.1	18.7	4.9	13.0		17.6	11.7
	1.3 = 8 per cent body weight loss		Totals......		(20)	40.0	(31)	25.7	23	35.2	25.4
8	14.3	Casein + basal	166	91	6.1	1.3	4.7	0		26.8	13.6
9	13.6	Casein + basal	123	81	6.0	10.5	5.8	7.8		19.8	13.5
	0.6 = 4 per cent body weight loss		Totals......		(9)	11.8	(13)	7.8	8		

Experimental History—Tables 3, 6, and 8.

Dog 40-34. Male bull. Born August, 1940. Continuous anemia history Apr. 20, 1942, to Feb. 9, 1943. Regular anemia experiments. Beginning weight 23.8 kilos. Blood volume 2170 cc., plasma volume 1014 cc. Feb 9, 1943—Daily diet of protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 400 mg. Plasma protein depletion begun. Regular double depletion experiments with interspersed recovery periods. May 1, 1944—Daily diet of protein-free basal biscuit 400 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Blood protein depletion begun. Plasma volume 1010 cc. Weight 21.2 kilos. A/G ratio 1.4.

May 26—Amino acid mixture Vuj *minus dl-phenylalanine* by vein or subcutaneously (Table 3). Daily diet of protein-free basal biscuit 250 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Periods 2 to 6. Plasma volumes 924 cc., 882 cc., 858 cc., 850 cc. A/G ratios 2.1, 0.7, 0.99, 1.5. June 22—Periods 6 and 7, *dl*-phenylalanine added to amino acid mixture. Daily diet as of May 26th. Plasma volumes 803 cc., 812 cc. A/G ratios 1.4, 0.9. July 8 — Dog in good ocondition other than weight loss. Periods 8 and 9. Daily diet of *casein* 35 gm., protein-free basal biscuit 200 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Plasma volumes 867 cc., 810 cc. A/G ratios 0.96, 0.80. July 21—Kennel diet and recovery period. Jan. 11, 1945—Daily diet of protein-free basal biscuit 200 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Blood protein depletion begun. Weight 20.9 kilos. Blood volume 1895 cc., plasma volume 870 cc. Plasma protein 6.0 gm per cent.

Feb. 16, 1945—Amino acid mixture Vuj *minus dl-isoleucine* by mouth (Table 6). Periods 2 to 5. Amino acids incorporated into basal biscuit. Daily diet of amino acid biscuit 220 gm., synthetic vitamin mixture 10 cc., reduced iron 600 mg. Plasma volumes 864 cc., 787 cc., 792 cc. A/G ratios 0.61, 0.75, 0.89. Period 5. *dl*-Isoleucine is incorporated into the amino acid mixture in the biscuit and fed for 9 days. Mar . 17—Experiment is terminated because of poor food consumption and general condition of the animal. Plasma volume 714 cc. A/G ratio 0.7. Period 6. Daily diet of salmon bread 200 gm., pig liver 300 gm., skim milk powder (Klim) 25 gm. Plasma volume 732 cc. Mar. 23—Marked improvement and gain in weight. Daily diet of kennel food and recovery period. Feb. 15, 1946—Blood protein depletion begun. Daily diet of protein-free basal biscuit 450 gm., canned carrots 200 gm., synthetic vitamin mixture 10 cc., choline chloride 600 mg. Blood volume 2170 cc., plasma volume 1014 cc. A/G ratio 1.1, plasma protein 6.3 gm. per cent. Weight 23.8 kilos.

Mar. 8, 1946—Amino acid mixture Vuj *minus dl-valine*, oral and subcutaneous (Table 8). Amino acid mixture is incorporated into the biscuit and is supplemented by subcutaneous amino acid administration. Daily diet of amino acid biscuit 350 gm., synthetic vitamin mixture 10 cc., choline chloride 600 mg. Periods 2 to 5. Plasma volumes 1031 cc., 982 cc., 909 cc. A/G ratios 1.0, 0.97, 0.87. Periods 5 to 7. *dl*-Valine is incorporated into the amino acid mixture in the biscuit. Because of poor food consumption amino acid mixture is given subcutaneously. Period 6. *Folic acid* 20 mg. given subcutaneously 3 times per week. Plasma volumes 968, 893 cc. A/G ratios 1.2, 1.4. Apr. 12—Experiment terminated. Dog in good condition other than weight loss. Period 7. Daily diet of canned salmon 170 gm., protein-free basal biscuit 300 gm., yeast 3 gm., liver extract powder 2 gm., choline chloride 600 mg. Food consumption 100 per cent. Plasma volume 940 cc. A/G ratio 1.0. Rapid gain in weight.

Table 4 presents two experiments with exclusion and replacement of *tryptophane* in the amino acid mixture. Both experiments are much alike. When tryptophane is omitted from the amino acid mixture, there develops a rapid weight loss, a large blood protein output, and some excess of urinary nitrogen but not enough to give a negative urinary nitrogen blance. When tryptophane is replaced in the amino acid mixture, it does not check the weight loss, the high blood protein output is maintained or increased, and there is some decrease in the urinary nitrogen.

There is a significant change in the *production ratio* of hemoglobin to plasma protein when tryptophane is returned to the amino acid mixture (periods 5, 6, and 7 in both experiments). There is actually more plasma protein produced than hemoglobin, in corrected figures. The preponderance of hemoglobin production in these double depletion experiments is an invariable reaction to all protein diets however we choose to explain it. When we note a preponderance of plasma protein production in dogs given tryptophane following a period of tryptophane exclusion, it invites speculation and suggests some special influence on the protein flow toward plasma protein synthesis. We note a steady fall in the hemoglobin levels and a definite rise in the plasma protein levels (periods 5 to 7, Table 4)—this means great differences between the hemoglobin actually removed and the net production of hemoglobin as the corrections

TABLE 4

Amino Acid Growth Mixture Minus Tryptophane—Oral, Subcutaneous, and Vein Blood Protein Output—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 42-1									
1	21.4	Basal	17	99	7.7	36.0	4.4	14.3		2.7	11.7
2	20.1	Amino a. − tryptophane	199	81	9.8	28.4	4.6	11.3		30.8	21.6
3	19.2	Amino a. − tryptophane	178	60	9.6	34.9	4.4	11.9		27.5	23.6
4	18.1	Amino a. − tryptophane	160	43	9.6	2.1	4.3	0		24.7	20.0
	3.3 = 15 per cent body weight loss	Totals.....			(84)	65.4	(17)	23.2	19	83.0	65.2
5	17.3	Amino a. + tryptophane	138	38	9.0	47.4	5.0	20.1		21.3	11.5
6	16.4	Amino a. + tryptophane	173	44	7.7	41.8	5.3	25.6		26.7	13.5
7	15.1	Amino a. + tryptophane	170	40	5.2	31.1	5.8	25.2		26.2	18.2
	3.0 = 17 per cent body weight loss	Totals.....			(47)	120.3	(85)	70.9	27	74.2	43.2
8	15.7	Yeast digest "Basamin"	187	100	5.2	1.0	4.8	0		30.0	13.2
		Dog 40-36									
1	17.0	Basal	18	95	8.6	26.5	4.3	11.5		2.9	
2	15.8	Amino a. − tryptophane	129	59	7.1	37.5	4.4	17.0		19.9	18.3
3	15.7	Amino a. − tryptophane	149	40	7.1	1.6	4.2	0		23.0	11.8
4	15.0	Amino a. − tryptophane	116	24	9.5	11.8	4.4	5.7		17.9	
	2.0 = 12 per cent body weight loss	Totals.....			(60)	50.9	(30)	22.7	23		
5	14.6	Amino a. + tryptophane	127	41	7.6	36.0	4.5	16.9		19.6	16.8
6	14.0	Amino a. + tryptophane	143	42	6.7	21.1	4.7	13.7		22.2	10.9
7	13.5	Amino a. + tryptophane	143	41	5.7	13.1	4.9	6.5		22.2	6.6
	1.5 = 10 per cent body weight loss	Totals			(29)	70.2	(42)	37.1	17	64.0	34.3
8	14.0	Salmon	254	96	8.7	25.5	4.6	12.6		40.7	5.0
9	14.1	Salmon	265	100	8.2	26.2	4.4	11.9		42.4	15.0
	0.6 = 4 per cent body weight gain	Totals......			(76)	51.7	(18)	24.5	18	83.1	20.0

are large because of the shrinkage in circulating hemoglobin. Just why tryptophane should favor plasma protein production at the expense of hemoglobin is not clear but deserves more study in this type of experiment.

On the basis of the corrected blood protein production figures the restoration of threonine and phenylalanine, as well as tryptophane is associated with a varying increase of plasma protein over hemoglobin. The restoration of missing basic amino acids, on the contrary, is associated with increased hemoglobin production. This latter response surely reflects the high content of arginine, histidine, and lysine in hemoglobin.

The after periods are of interest. Salmon protein promptly corrects weight loss, increases hemoglobin production, and improves nitrogen balance. Yeast digest protein or protein-forming materials (dog 42-1) check weight loss and improve nitrogen balance but show no evidence in 1 week of new blood protein formation.

Tryptophane in other experiments (Table 3 (2)) dealing with plasma protein production in sustained hypoproteinemia, showed a definite response. When tryptophane was omitted from the growth mixture of amino acids, there was a fall in plasma protein production and weight loss. The replacement of tryptophane in the mixture gave a slow return toward the expected levels of plasma protein production.

Experimental History—Tables 4 and 6.

Dog 42-1. Male bull adult. Continuous anemia history Feb. 14, 1945, to Aug. 8, 1945. Regular anemia experiments. Beginning weight 17.7 kilos, blood volume 1388 cc., plasma volume 738 cc. Aug. 8—Daily diet of protein-free basal biscuit 450 gm., yeast 5 gm., liver extract powder 2 gm. Depletion of plasma proteins begun. Blood volume 1439 cc., plasma volume 964 cc. Weight 19.1 kilos. Plasma proteins 6.1 gm. per cent.

Aug. 31—Amino acid mixture Vuj *minus dl-isoleucine*, oral and subcutaneous (Table 6). Amino acids incorporated into basal biscuit. Periods 2 to 5 . Daily diet amino acid biscuit 350 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Plasma volumes 900 cc., 829 cc., 852 cc. A/G ratios 0.83, 0.74, 0.84. Periods 5 and 6. *dl*-Isoleucine is incorporated into the amino acid mixture in the biscuit. Daily diet amino acid biscuit 275 gm., synthetic vitamin mixture 10 cc. Slight edema right front leg. Traumatic skin lesions on joints. Amino acid biscuit feeding supplemented by similar amino acid mixture given subcutaneously to increase nitrogen intake. Synthetic vitamin mixture increased to 15 cc. Period 7. Trace of bile pigment in plasma. Leucocytes average 20,000 per cubic mm. Oct. 4—Skin lesions not improved. Edema of right front foot not imporoved. Periods 5 to 7 inclusive. Plasma volumes 776 cc., 792 cc., 720 cc. A/G ratios 0.84, 0.93, 0.84. Period 7. Daily diet pig liver 200 gm., protein-free basal biscuit 375 gm., yeast 5 gm., liver extract powder 3 gm. Oct. 19—Experiment terminated. Moderate improvement of skin lesions. Oct. 19 to Feb. 20, 1946—Recovery period. Daily diet of salmon bread and liver. Feb. 20—Daily diet of protein-free basal biscuit 400 gm., canned onions 150 gm., liquid vitamin mixture 10 cc., kennel diet 100 gm. Depletion of blood proteins begun. Beginning blood volume 1785 cc., plasma volume 1052 cc., weight 23.4 kilos, plasma protein 6.6 gm. per cent. Mar. 2—Daily diet of protein-free basal biscuit 450 gm., canned onions 200 gm., liquid vitamin mixture 10 cc.

Mar. 15—Amino acid mixture Vuj *minus dl-tryptophane* oral, subcutaneous or vein (Table 4). Amino acids incorporated into basal biscuit. Periods 2 to 5. Daily diet of amino acid biscuit 300 gm., liquid vitamin mixture 10 cc., choline chloride 600 mg. Amino acid is supplemented by amino acid mixture given subcutaneously or by vein. Plasma volumes 1045 cc., 960 cc., 928 cc., A/G ratios 1.1, 0.99, 1.2. Apr. 5—Periods 5 to 8, *dl*-tryptophane is incorporated into the amino acid mixture in the biscuit. Daily diet of amino acid biscuit 200 gm., liquid vitamin mixture 10 cc., choline chloride 300 mg. Amino acid feeding is supplemented by amino acids given subcutaneously or by vein. Plasma volumes 852 cc., 850 cc., 795 cc. A/G ratios 1.1, 1.2, 1.0. Period 8. Daily diet of yeast digest (Basamin) 35 gm., protein-free basal biscuit 300 gm., yeast 3 gm., liver extract powder 2 gm. Plasma volume 924 cc. A/G ratio 1.6.

Experimental History—Table 4.

Dog 40-36. Male bull. Born August, 1940. Continuous anemia history Jan. 20, to Sept. 8, 1944. Regular anemia experiments. Beginning weight 14.1 kilos, blood volume 1278 cc., plasma volume 627 cc. Sept. 8, 1944—Daily diet of protein-free basal biscuit 500 gm., yeast 5 gm., reduced iron 600 mg. Plasma protein depletion begun. Regular double depletion experiments with interspersed recovery periods. Jan. 23, 1946—Daily diet of protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm. Blood protein depletion begun. Plasma volume 844 cc., weight 20.3 kilos A/G ratio 1.5.

Feb. 15, 1946—Amino acid mixture Vuj *minus dl-tryptophane* oral and by vein (Table 4). Amino acids incorporated into basal biscuit. Daily diet of amino acid biscuit 340 gm., synthetic vitamin mixture 5 cc., dextrose 50 gm. Periods 2 to 5. Plasma volumes 810 cc., 920 cc., 760 cc. A/G ratios 0.98, 1.0, 0.94. Period 4. Amino acid mixture given subcutaneously. Mar. 8—Periods 5 to 8, *dl*-tryptophane is incorporated into the amino acid mixture in the biscuit and fed. Amino acid administration is supplemented by injection. Daily diet of amino acid biscuit 200 gm., protein-free basal biscuit 150 gm., synthetic vitamin mixture 5 cc., choline chloride 600 mg. Periods 5 to 8. Plasma volumes 749 cc., 800 cc., 771 cc. A/G ratios 1.1, 1.1, 1.1. Mar. 29—Periods 8 and 9, daily diet of canned salmon 130 gm., protein-free basal biscuit 400 gm., yeast 3 gm., choline chloride 600 mg. Plasma volumes 724 cc., 712 cc. A/G ratios 1.2, 1.2. Dog in good condition.

Table 5 (leucine) shows three experiments on two dogs which add the factors of *disease* to the general response. The first experiment (dog 37-85) shows a satisfactory period of amino acid mixture *minus leucine* given by mouth. The urinary nitrogen balance is positive, the new blood protein output net is 86 gm., and there is considerable weight loss (15 per cent in 3 weeks) in spite of adequate intake of the protein-free diet. The blood protein output per kilo weight loss is 33 gm. A high protein diet intake (periods 5 and 6) shows a prompt response with gain in weight and high blood protein production. Experimental history of this dog (37-85) follows Table 2.

The second dog (43-250) shows a totally different response and the autopsy shows abnormalities which we believe explain at least in part some of the unusual figures. The first experiment on dog 43-250 is not according to the expected pattern—there is a negative nitrogen balance in all periods of amino acid by vein with and without leucine. This dog appeared essentially normal but was purchased from a dealer and the past history unknown—a mongrel hound of several years of age. The dog was in our kennels for 2 years and various double depletion experiments were carried out, followed by rest periods. There was a 4 per cent weight loss per week on amino acids minus leucine and a slight gain in weight during 1 week when leucine was added to the mixture. The output of blood proteins was insignificant. We suspect that at this time this dog may have had some bronchopneumonia and some renal abnormality—see autopsy below. Recovery followed on kennel diet.

At a later period dog 43-250 (Table 5) was again tested on the amino acid mixture minus and plus leucine by vein. In this experiment the nitrogen output in the urine was high and the weight loss was extreme (15 per cent in 3 weeks). The blood protein output was not large but was definite and probably in part was related to the weight loss. *Autopsy*—The dog died on the 3rd day following period 4 (Table 5) and showed conspicuous abnormalities of the lungs. Pulmonary emboli or thrombi were found—patches of bronchopneumonia were numerous, some acute and others of some weeks' standing—terminal pulmonary edema. The kidneys showed some chronic thickening of the glomerular tufts but the tubules and stroma were normal. Other findings negative.

Obviously the high urinary nitrogen and rapid weight loss are explained in part by the pulmonary abnormalities. We may suspect that a similar but less extensive pulmonary infection may have been present in the first experiment in this dog (Table 5) followed by recovery. It is of considerable interest that this dog continued to produce blood proteins in the face of a respiratory infection and obviously there was loss of body protein, probably some of it going to form needed hemoglobin and plasma protein.

Leucine omission from the mixture of essential amino acids has been reported (Table 3 (2)) in the study of new plasma protein production in hypoproteinemia. Plasma protein production was ample but there was a large excess of urinary nitrogen and weight loss due to the deletion of leucine.

Experimental History—Table 5.

Dog 43–250. Male black and tan hound maintained in laboratory kennels for several months under optimum conditions. Feb. 23, 1944—Blood protein depletion begun. Daily diet of protein-free basal biscuit 450 gm., yeast 5 gm., reduced iron 600 mg. Beginning blood volume 1560 cc., plasma volume 758 cc., weight 18.0 kilos. Regular double depletion experiments with interspersed recovery periods. Apr. 13, 1944—Daily diet desiccated horse liver (Arlington Chemical Co.) 75 gm., protein-free basal biscuit 300 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Plasma volume 816 cc. A/G ratio 1.0.

Apr. 28—Amino acid mixture Vuj *minus l* (−) *leucine* by vein (Table 5). Daily diet of protein-free basal biscuit 350 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Periods 2 and 3. Plasma volume 852 cc. and 892 cc. A/G ratio 1.8 and 1.5. Period 4. *l* (−) Leucine added to amino acid mixture. Daily diet as of Apr. 28. Plasma volume 895 cc. A/G ratio 1.1. Kennel diet and recovery period. Aug. 9, 1944—Blood protein depletion begun. Regular double depletion experiments. Jan. 4, 1946— Daily diet of protein-free basal biscuit 400 gm., yeast 3 gm., liver extract powder 2 gm. Plasma volume 954 cc. A/G ratio 1.0.

Jan. 11—Amino acid mixture Vuj *minus l* (−) *leucine* by vein or subcutaneously (Table 5). Daily diet of protein-free basal biscuit 350 gm., synthetic vitamin mixture 10 cc. Periods 2 and 3. Plasma volumes 912 cc. and 870 cc. A/G ratios 2.2 and 1.5. Period 4. *l* (−) Leucine added to vitamin mixture. Daily diet as of Jan. 11. Plasma volume 888 cc. A/G ratio 1.2. Feb. 4—Dog left 85 per cent of his food, is apathetic, and appears ill. Feb. 5—Dog found dead. Autopsy summary above.

Table 6 shows two experiments to indicate that *isoleucine* can be spared from the amino acid mixture for 3 weeks without significant disturbance of urinary nitrogen balance and blood protein output. A strong positive urinary nitrogen balance is noted in dog 42-1 (Table 6) whether isoleucine is present or absent from the standard amino acid mixture.

Dog 42-1 (Table 6). The weight loss is large (6 per cent per week) when isoleucine is deleted from the amino acid mixture (periods 2 to 4) and the blood protein net output (100 gm.) is correspondingly high. When isoleucine is replaced in the amino acid mixture there is less weight loss per week and the blood protein output continues at the same high level—net figures 67 gm. per 2 weeks. Heavy feeding with liver corrects the loss of weight and increases the net blood protein output (50 gm. per week).

TABLE 5

Amino Acid Growth Mixture Minus Leucine—Intravenous and Oral Blood Protein Output—Basal Diet in All Periods—Autopsy

Period	Weight	Protein intake		Food consumption	Protein output				Production ratio net protein output to intake	Total nitrogen weekly	
		Type	Weekly		Hemoglobin		Plasma protein			Intake	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 37-85 (mouth)									
1	17.8	Casein	103	93	6.3	26.8	4.9	14.0		16.5	—
2	17.0	Amino a. — leucine	73	78	6.3	22.7	5.4	15.4		11.5	9.2
3	16.4	Amino a. — leucine	83	89	6.9	26.0	5.3	16.0		13.2	10.1
4	15.2	Amino a. — leucine	84	90	6.9	13.5	4.9	8.0		13.2	8.4
	2.6 = 15 per cent body weight loss	Totals.........			(56)	62.2	(30)	39.4	36	37.9	27.7
5	16.4	Liver	764	100	11.8	47.8	5.3	26.7		123	—
6	17.4	Liver	764	100	12.3	57.1	5.5	25.3		123	
	2.2 = 14 per cent body weight gain	Totals.......			(198)	104.9	(58)	52.0	17		
		Dog 43-250 (vein)									
1	15.7	Liver	429	100	7.6	32.2	5.7	19.9		53.0	—
2	14.9	Amino a. — leucine	93	80	6.0	19.2	5.4	12.6		14.8	17.4
3	14.5	Amino a. — leucine	77	62	6.0	1.3	5.0	0		12.4	13.6
	1.2 = 8 per cent body weight loss	Totals..........			(0)	20.5	(9)	12.6	2	27.2	31.0
4	14.6	Amino a. + leucine	109	82	6.6	1.2	4.9	0		16.8	18.1
		Dog 43-250 Terminal infection—autopsy									
1	18.4	Basal	15	79	10.0	2.0	4.1	0		2.4	7.1
2	18.0	Amino a. — leucine	94	69	9.2	32.0	4.5	14.9		15.6	13.1
3	17.0	Amino a. — leucine	94	55	7.1	37.1	5.0	21.4		15.6	21.6
	1.4 = 8 per cent body weight loss	Totals........			(21)	69.1	(41)	36.3	33	31.2	34.8
4	15.8	Amino a. + leucine	108	49	5.5	18.5	6.3	15.5		17.3	14.7
	1.2 = 7 per cent body weight loss	Totals........			(0)	—24	(23)	15.5	16		

Experimental history of this dog (42-1) follows Table 4. Dog 40-34 (Table 6) shows much the same response although a less complete experiment.

Observations have been reported recently (Table 3 (2)) to show that amino acid growth mixtures *minus isoleucine* in hypoproteinemic dogs precipitated an increase in urinary nitrogen and a fall in plasma protein output. The output of plasma protein increased when isoleucine was replaced in the mixture but the nitrogen balance required several weeks to get back to the control level. Experimental history of this dog 40-34 follows Table 3.

TABLE 6

Amino Acid Growth Mixture Minus Isoleucine—Oral and by Injection
Blood Protein Output—Basal Diet in All Periods

Period	Weight	Protein intake		Food consumption	Protein output				Production ratio net protein output to intake	Total nitrogen weekly	
		Type	Weekly		Hemoglobin		Plasma protein			Intake	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 42-1									
1	18.1	Basal	19	100	8.5	1.7	4.2	0		3.0	11.2
2	17.1	Amino a. − isoleucine	132	100	9.1	48.5	4.7	20.4		20.3	9.3
3	16.0	Amino a. − isoleucine	118	92	7.4	41.9	4.5	21.8		18.2	11.2
4	14.7	Amino a. − isoleucine	80	66	6.5	10.6	4.0	4.6		12.3	9.7
	3.4 = 19 per cent body weight loss	Totals........			(60)	101.0	(40)	46.8	30	50.8	30.2
5	14.2	Amino a. + isoleucine	127	76	7.1	21.4	5.1	14.1		19.9	9.5
6	13.7	Amino a. + isoleucine	122	55	7.1	11.2	5.4	7.3		18.8	10.4
	1.0 = 7 per cent body weight loss	Totals.........			(37)	32.6	(30)	21.4	27	38.7	19.9
7	14.1	Liver	321	98	9.6	20.3	5.5	12.0		—	—
		Dog 40-34 (oral)									
1	16.8	Basal	17	88	6.2	11.2	4.5	7.2			
2	15.6	Amino a. − isoleucine	161	69	7.8	20.0	5.3	14.4			
3	14.8	Amino a. − isoleucine	213	91	7.4	23.1	4.8	14.0			
4	14.1	Amino a. − isoleucine	128	55	7.2	14.5	4.2	7.3			
	2.7 = 16 per cent body weight loss	Totals........			(55)	57.6	(20)	35.7	15		
5	13.2	Amino a. + isoleucine	119	47	7.3	13.1	5.1	7.2			
	0.9 = 6 per cent body weight loss	Totals.........			(7)	13.1	(10)	7.2	14		
6	14.1	Liver	667	100	8.9	1.2	5.8	0			

Table 7 indicates that *lysine* has only a modest effect upon nitrogen metabolism. Dog 40-32 tolerated a 5 week period on growth amino acids minus lysine by vein with a uniform response. The production of blood proteins is sustained and averages about 20 gm. per week—a total net output of 114 gm. or 60 gm. per kilo weight loss. The nitrogen intake and urinary output are about in balance. Diet intake of the basal biscuit is excellent.

Dog 41-52 (Table 7) shows a slight effect when the lysine is replaced in the amino acid mixture (periods 6 and 7). There is improvement in nitrogen retention, considering the increased intake of nitrogen but the blood protein output decreases and the weight loss continues. The output of blood proteins in the four periods minus lysine (periods 2 to 5) is high—total net output of 159 or 40 gm. per week or 51 gm. per kilo weight loss. The question of raiding of body proteins to form needed blood protein comes into the discussion. The final week of heavy liver feeding reverses promptly the weight loss and increases about fourfold the blood protein output.

TABLE 7

Amino Acid Growth Mixture Vuj Minus Lysine—Oral, Subcutaneous, and Vein Blood Protein Output—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 40-32									
1	18.8	Basal	72	100	10.2	16.1	4.5	6.9		11.5	—
2	18.8	Amino a.—lysine	95	100	9.9	18.9	5.1	8.5		14.5	14.6
3	18.4	Amino a.—lysine	95	100	9.9	25.4	4.7	12.0		14.5	14.8
4	17.9	Amino a.—lysine	95	93	7.5	33.4	4.6	17.5		14.5	14.4
5	17.6	Amino a.—lysine	95	93	8.2	23.5	4.5	13.6		14.5	12.4
6	16.9	Amino a.—lysine	95	90	7.5	9.1	4.1	4.5		14.5	12.2
	1.9 = 10 per cent body weight loss	Totals........			(65)	110.3	(49)	57.0	24	72.5	68.4
		Dog 41-52 (oral, subcutaneous, and vein)									
1	17.3	Basal	19	100	9.1	16.1	4.5	6.3		3.0	12.1
2	16.9	Amino a.—lysine	106	90	10.8	58.6	4.4	19.2		15.9	9.5
3	16.1	Amino a.—lysine	89	77	8.3	39.7	4.7	18.8		13.7	12.0
4	14.6	Amino a.—lysine	79	57	8.9	19.8	4.6	9.4		12.1	11.0
5	14.2	Amino a.—lysine	166	73	8.0	25.4	4.6	10.9		25.4	14.3
	3.1 = 18 per cent body weight loss	Totals........			(105)	143.5	(54)	58.3	36	67.1	46.8
6	13.8	Amino a. + lysine	152	52	8.0	15.1	4.3	6.4		23.5	12.4
7	12.7	Amino a. + lysine	134	41	9.7	1.9	4.8	0		20.8	10.4
	1.5 = 11 per cent body weight loss	Totals........			(27)	18.0	(3)	6.4	10	44.3	22.8
8	13.7	Liver	469	99	8.0	54.0	5.3	28.3		75.0	36.7

Experimental History—Tables 9, 7, 10, and 8.

Dog 40-32. Male bull. Born August, 1940. Continuous anemia history Dec. 2, 1941, to May 26, 1942. Regular anemia experiments. Beginning weight 16.5 kilos, blood volume 1400 cc. May 26, 1942—Daily diet of protein-free basal biscuit 350 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 400 mg. Plasma protein depletion begun. Regular double depletion experiments with interspersed recovery periods.

Feb. 3, 1944—Amino acid mixture Vuj *minus l* (+) *arginine HCl* by vein (Table 9). Blood volume 1301 cc., plasma volume 994 cc. Daily diet of protein-free basal biscuit 400 gm., synthetic vitamin mixture 5 cc., reduced iron 600 mg. Periods 2 to 5. Plasma volumes 958 cc., 888 cc., 997 cc. A/G ratios 1.15, 1.18, 1.07. Feb. 24—Periods 5 to 7 inclusive, *l* (+) arginine HCl was added to the amino acid mixture administered by vein. Diet as of Feb. 3. Plasma volumes 879 cc., 936 cc., 860 cc. A/G ratios 1.3, 1.2, 1.1. Mar. 16—Experiment terminated. Dog in good condition other than weight loss. Kennel diet and recovery period. June 1, 1944—Blood protein depletion begun. Blood volume 1486 cc., plasma volume 954 cc. Plasma protein 6.4 gm. per cent. Daily diet of protein-free basal biscuit (containing some barley) 400 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Food consumption 100 per cent.

June 16—Amino acid mixture Vuj *minus l* (+) *lysine HCl* by vein (Table 7). Period 1. Daily diet of basal biscuit containing barley 400 gm., yeast 3 gm., liver extract powder 2 gm. Blood volume 1390 cc., plasma volume 990 cc. Periods 2 to 6 inclusive. Daily diet protein-free basal biscuit 400 gm., synthetic vitamin mixture 8 cc., reduced iron 600 mg. Amino acid mixture given by vein. Plasma volumes 990 cc., 954 cc., 928 cc., 974 cc., 926 cc. A/G ratios 0.95, 1.6, 1.0, 0.98, 1.1. July 21—Experiment terminated. Dog in good condition. Kennel diet and recovery period. Oct. 17— Blood protein depletion begun. Blood volume 1584 cc., plasma volume 958 cc., weight 20.1 kilos. Plasma protein 5.4 gm. per cent. Daily diet of protein-free basal biscuit 400 gm., barley mush 300 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg.

Nov. 3—Amino acid mixture Vuj *minus l* (+) *histidine HCl* by vein (Table 10). Periods 2 to 6. Daily diet of protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Amino acid mixture given by vein. Plasma volumes 1065 cc., 1054 cc., 1050 cc., 1010 cc. A/G ratios 1.4, 1.4, 1.0, 1.3. Dog in good condition throughout experiment. Periods 6 to 9 inclusive. Daily diet of whole egg 120 gm., protein-free basal biscuit 400 gm., yeast 3 gm., liver extract powder 2 gm., synthetic vitamin mixture 10 cc., reduced iron 600 mg. Plasma volumes 1010 cc., 1040 cc., 988 cc., 1040 cc. A/G ratios 1.3, 1.2, 1.1, 0.86. Moderate skin lesions. Dec. 29—Experiment terminated. Dec. 29 to Feb. 9, 1945—High protein diet. Double depletion experiments continued.

May 18, 1945—Amino acid mixture Vuj *minus dl-valine,* oral and vein (Table 8). Periods 2 to 5 . Amino acid mixture incorporated into basal biscuit. Daily diet of amino acid biscuit 325 gm., yeast 3 gm., liver extract powder 2 gm. Periods 3 to 5 . Amino acid biscuit feeding supplemented by amino acid mixture given by vein. Periods 2 to 5. Plasma volumes 967 cc., 829 cc., 888 cc. A/G ratios 0.89, 0.99, 1.1. Periods 5 and 6. Daily diet of liver 87 gm., lean beef 57 gm., salmon bread 85 gm., Klim 5 gm., protein-free basal biscuit 350 gm., yeast 3 gm. Plasma volumes 966 cc., 906 cc. A/G ratios 1.1, 0.95. June 29—Kennel diet and recovery period.

Experimental History—Table 7.

Dog 41-52. Male bull adult. Maintained in laboratory kennels for several months under optimum conditions. Aug. 8, 1945—Daily diet of protein-free basal bisucuit 400 gm., salmon bread 75 gm., yeast 3 gm., liver extract powder 2 gm. Blood protein depletion begun. Beginning weight 18.4 kilos, blood volume 1579 cc., plasma volume 742 cc., plasma protein 6 gm. per cent. Aug. 30—Salmon bread omitted from diet.

Aug. 31—Amino acid mixture Vuj *minus l* (+) *lysine HCl* oral and parenterally (Table 7). Periods 2 to 4. Amino acids incorporated into basal biscuit. Daily diet of amino acid biscuit 337 gm., synthetic vitamin mixture 10 cc. Sept. 7—Period 3, dextrose 20 gm. added to daily diet. Sept. 21—Periods 4 and 5, amino acid biscuit feeding supplemented by amino acids given parenterally. Periods 2 to 6. Plasma volumes 793 cc., 803 cc., 756 cc., 736 cc. A/G ratios 0.93, 0.93, 0.8, 0.72. Sept. 28—Periods 6 and 7, *l* (+) lysine HCl is incorporated into the amino acid mixture in the biscuit. Daily diet of amino acid biscuit 275 gm., synthetic vitamin mixture 15 cc., sugar mixture 50 gm. Amino acid feeding supplemented by parenteral administration. Periods 6 and 7. Plasma volumes 744 cc., 649 cc. A/G ratios 0.76, 0.68. Dog in good condition other than weight loss. Oct. 11—Period 8, daily diet of liver 300 gm., salmon bread 100 gm., protein-free basal biscuit 100 gm., yeast 3 gm., liver extract powder 2 gm. Regular double depletion experiments to Dec. 13, 1945. Rapid gain of weight.

TABLE 8

Amino Acid Growth Mixture Minus Valine—Oral, Vein, and Subcutaneous Blood Protein Output—Basal Diet in All Periods

Period	Weight	Protein intake		Food consumption	Protein output				Production ratio net protein output to intake	Total nitrogen weekly	
		Type	Weekly		Hemoglobin		Plasma protein			Intake	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 43-174									
1	18.3	Basal	19	100	8.9	29.3	4.4	11.6		3.0	13.1
2	18.2	Amino a. − valine	68	56	9.9	28.7	4.4	12.5		11.0	20.3
3	16.4	Amino a. − valine	105	64	10.2	29.5	4.5	11.8		17.3	15.3
	1.9 = 10 per cent body weight loss	Totals........			(52)	58.2	(17)	24.3	40	28.3	35.7
4	15.3	Amino a. + valine	123	74	8.6	38.6	4.5	15.5		19.0	15.2
5	14.7	Amino a. + valine	112	88	7.7	13.7	4.1	6.4		17.3	13.4
	1.7 = 10 per cent body weight loss	Totals........			(18)	52.3	(19)	21.9	16	36.3	28.6
		Dog 40-32									
1	18.7	Liver	301	100	8.1	34.8	5.3	16.4		44.2	17.1
2	17.7	Amino a. − valine	106	82	8.2	26.1	5.0	14.9		16.4	12.7
3	16.2	Amino a. − valine	125	71	7.7	31.5	4.7	15.7		19.3	11.7
4	15.6	Amino a. − valine	116	62	7.5	19.1	4.4	10.4		18.0	20.3
	3.1 = 17 per cent body weight loss	Totals........			(69)	76.7	(33)	41.0	29	53.7	44.7
5	17.2	Liver	301	100	8.1	21.6	4.6	11.0		44.2	15.9
6	17.2	Liver	301	100	8.6	28.5	4.5	12.7		—	—
	1.6 = 10 per cent body weight gain	Totals.......			(66)	50.1	(24)	23.7	15		
		Dog 40-34									
1	20.9	Basal	12	81	8.6	29.8	4.5	13.0		1.9	—
2	19.9	Amino a. − valine	127	40	7.7	25.0	4.4	12.4		19.5	9.7
3	18.7	Amino a. − valine	138	42	7.9	11.9	4.4	5.8		21.2	17.7
4	17.5	Amino a. − valine	118	20	5.6	24.2	4.8	13.9		18.1	19.3
	3.4 = 16 per cent body weight loss	Totals........			(8)	61.1	(29)	32.1	10	58.8	46.7
5	16.5	Amino a. + valine	115	8	5.6	1.2	5.2	0		17.8	18.0
6	15.4	Amino a. + valine	136	18	6.2	1.3	6.3	0		21.0	18.3
	2.1 = 12 per cent body weight loss	Totals........			(5)	2.5	(12)	0	—	38.8	36.3
7	17.2	Salmon	352	100	7.7	24.6	5.2	16.2		56.3	17.3

Table 8 shows three experiments to indicate that omission of *valine* from the amino acid growth mixture does not cause any notable change in the nitrogen balance and weight balance. Blood protein production goes on with or without valine.

Dog 43-174 (Table 8) is a satisfactory experiment and there is little change in weight loss which is considerable (5 per cent per week) with and without valine in the amino acid mixture. The nitrogen balance improves considerably when valine is replaced.

The blood protein net output is 69 gm. without valine and 37 gm. when valine is replaced. Food intake is quite satisfactory.

Dog 40-32 (Table 8) shows a 3 week period with valine deleted from the amino acid mixture. The nitrogen balance is positive, the blood protein net output is large (102 gm. in 3 weeks), and basal diet intake adequate. We do not attempt to explain the high urinary nitrogen in period 4. A large liver diet intake corrects the weight loss, increases the positive nitrogen balance, and causes an abundant blood protein output (90 gm. in 2 weeks). Experimental history of this dog (40-32) follows Table 7.

Dog 40-34 (Tabel 8) is a good experiment but low food consumption of the basal diet is probably in part responsible for the rather large urinary nitrogen output. The blood protein output is decidedly low in all periods. A liberal salmon diet corrects the weight loss and urinary nitrogen loss and increases the blood protein output. Experimental history of this dog (40-34) follows Table 3.

Other experiments with hypoproteinemic dogs and plasma protein production (Table 4 (1)) indicated that valine omission may decrease plasma protein production and result in negative N balance.

Experimental History—Table 8.

Dog 43–174. Female pointer adult. Maintained in laboratory kennels for several months under optimum conditions. Dec. 27, 1945—Daily diet of protein-free basal biscuit 400 gm., yeast 3 gm., liver extract powder 2 gm. Blood protein depletion begun. Beginning weight 18.9 kilos, blood volume 1666 cc., plasma volume 828 cc., plasma protein 6 gm. per cent. A/G ratio 1.5. Jan. 4, 1946—Protein-free basal biscuit increased to 450 gm. Plasma volume 911 cc.

Jan. 11—Amino acids mixture Vuj *minus dl-valine* oral (Table 8). Amino acids incorporated into basal biscuit. Daily diet of amino acid biscuit 350 gm., sugar mixture 100 gm., synthetic vitamin mixture 10 cc. Periods 2 and 3. Plasma volumes 956 cc., 852 cc. A/G ratios 1.7 and 1.4. Jan. 25, 1946—Periods 4 and 5, *dl*-valine is incorporated into the amino acid mixture in the biscuit. Daily diet of amino acid biscuit 425 gm., synthetic vitamin mixture in form of two capsules. Plasma volumes 762 cc., 764 cc. A/G ratios 1.6, 2.7. Feb. 9—Daily diet of kennel food and recovery period. Dog in good condition other than weight loss.

Table 9 (dog 40-32) shows a satisfactory experiment, 3 weeks with and 3 weeks without *arginine* in the amino acid mixture. The withdrawal of arginine appears to exert no influence upon the nitrogen excretion in the urine and a slight influence on weight loss. The blood protein production is somewhat higher when arginine is replaced in the amino acid mixture—52 gm. without and 87 gm. with arginine and the production of hemoglobin rises rapidly in periods 5 to 7. Experimental history of this dog (40-32) follows Table 7.

Table 9 (dog 40-33) shows experiments in which *arginine* and *histidine* are deleted from the amino acid mixture. The dog tolerates surprisingly well the withdrawal of both amino acids in periods 2 to 4, in fact the response is about what one would expect associated with administration of the complete amino acid mixture. The weight loss is slight and the blood protein output about a normal average. The urinary nitrogen is a bit increased. Replacement of histidine in periods 5 and 6 and then histidine plus arginine in periods 7 and 8 effects some nitrogen conservation and a positive urinary nitrogen balance. There is slightly more weight loss and a little more blood protein production in periods 7 and 8. Preponderance of regenerated hemoglobin compared with plasma protein is conspicuous in periods 7 and 8.

When casein replaces the amino acid mixture there is a prompt increase in nitrogen retention and weight loss is negligible.

TABLE 9

Amino Acid Growth Mixture Minus Arginine—Intravenous Blood Protein Output—Basal Diet in All Periods

Period	Weight	Protein intake		Food consumption	Protein output				Production ratio net protein output to intake	Total nitrogen weekly	
		Type	Weekly		Hemoglobin		Plasma protein			Intake	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 40–32									
1	17.2	Basal	19	100	6.8	11.0	4.6	6.4		3.0	6.0
2	17.0	Amino a. − arginine	97	86	6.6	12.0	4.6	6.6		15.5	8.6
3	16.7	Amino a. − arginine	97	90	5.7	23.3	5.4	15.8		15.5	8.8
4	16.0	Amino a. − arginine	97	68	5.7	1.2	5.5	0		15.5	9.9
	1.2 = 8 per cent body weight loss	Totals.........			(21)	36.5	(31)	22.4	18	46.5	27.3
5	15.7	Amino a. + arginine	107	78	6.0	18.1	5.2	11.5		17.1	10.6
6	15.2	Amino a. + arginine	107	74	6.0	1.3	5.4	0		17.1	10.6
7	15.1	Amino a. + arginine	107	85	7.3	20.3	5.4	14.2		17.1	10.9
	0.9 = 6 per cent body weight loss	Totals.........			(52)	39.7	(35)	25.7	27	51.3	32.1
		Dog 40–33 Amino acid growth mixture minus histidine and arginine—intravenous									
1	17.8	Basal	19	100	8.6	1.3	4.2	0		3.0	—
2	17.8	Amino a. − histidine-arginine	89	87	8.1	26.8	4.5	12.9		14.2	12.6
3	17.7	Amino a. − hist.-arg.	89	99	5.4	29.7	4.7	17.2		14.2	14.5
4	17.1	Amino a. − hist.-arg.	89	78	6.4	5.2	4.7	6.3		14.2	14.1
	0.7 = 4 per cent body weight loss	Totals.........			(34)	61.7	(44)	36.4	29	42.6	41.2
5	16.7	Amino a. + hist.-arg.	97	85	5.9	36.8	5.2	21.1		15.5	11.5
6	15.9	Amino a. + hist.-arg.	97	73	5.9	1.2	5.0	0		15.5	12.4
	1.2 = 7 per cent body weight loss	Totals.........			(28)	38.0	(21)	21.1	25	31.0	23.9
7	15.1	Amino a. + hist. + arg.	107	74	7.9	12.9	5.7	8.8		17.1	13.7
8	14.6	Amino a. + hist. + arg.	107	92	7.8	19.1	5.1	13.0		17.1	12.6
	1.3 = 8 per cent body weight loss	Totals.........			(55)	32.0	(15)	21.8	33	34.2	26.3
9	14.5	Casein	190	93	7.8	21.3	5.2	12.6		30.4	7.2
10	14.7	Beef	248	100	8.7	35.7	5.4	19.0		39.7	11.7

Experimental History—Table 9.

Dog 40–33. Male bull. Born August, 1940. Continuous anemia history Oct. 27, 1942, to June 2, 1943. Regular anemia experiments. Beginning weight 16.2 kilos. Blood volume

1340 cc., plasma volume 734 cc. June 2, 1943—Daily diet of protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Plasma protein depletion begun. Regular double depletion experiments with interspersed recovery periods. Dec. 29—Daily diet of protein-free basal biscuit 500 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Blood protein depletion begun. Plasma volume 866 cc. Weight 19.1 kilos. Plasma protein 6.4 gm. per cent.

Jan. 20, 1944—Amino acid mixture Vt *minus l* (+) *histidine HCl*, and *l* (+) *arginine HCl*, by vein (Table 9).

Vt amino acid mixture = *dl*-threonine 1.4 gm., *dl*-valine 3.0 gm., *l* (−) leucine 3.0 gm., *dl*-isoleucine 2.8 gm., *l* (+) lysine HCl 3.0 gm., *l* (−) trytptophane 0.8 gm., *dl*-methionine 1.2 gm., *dl*-phenylalanine 2.0 gm., glycine 2.0 gm., amino acid mixture given by vein.

Daily diet of protein-free basal biscuit 500 gm., synthetic vitamin mixture 5 cc., reduced iron 600 mg. Periods 2 to 5. Plasma volumes 972 cc., 910 cc., 997 cc. A/G ratios 1.6, 1.2, 1.0. Periods 5 and 6. *l* (+) Histidine HCl added to amino acid mixture given by vein. Daily diet of protein-free basal biscuit 350 gm., synthetic vitamin mixture 5 cc., reduced iron 600 mg. Plasma volumes 876 cc., 978 cc. A/G ratios 0.9, 1.0. Periods 7 and 8. *l* (+) Arginine HCl added to amino acid mixture given by vein. Daily diet of protein-free basal biscuit 300 gm., synthetic vitamin mixture 5 cc., reduced iron 600 mg. Plasma volumes 915 cc., 808 cc. A/G ratios 1.3, 1.4. Period 9. Dog in good condition. Daily diet of casein 30 gm., protein-free basal biscuit 350 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Plasma volume 826 cc. A/G ratio 1.1. March 16—Period 10, daily diet of lean beef 150 gm., protein-free basal biscuit 350 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Plasma volume 782 cc. A/G ratio 1.1. Experiment terminated, dog in excellent condition.

Table 10 (dog 40-32) again gives evidence that the dog under these conditions tolerates well the withdrawal of *histidine* from the amino acid mixture. There is a positive urinary nitrogen balance, a good production of blood proteins, but a considerable weight loss. Food consumption is excellent. When egg protein replaces the amino acid mixture in slightly greater amounts, there is a great change in the general picture—weight loss changed to weight gain—urinary nitrogen loss of 63 gm. changed to 37 gm. There is no significant difference in blood protein output but a reversal of the amounts of hemoglobin and plasma protein produced. Withdrawal of histidine shows more plasma protein and less hemoglobin production as compared with the egg protein. Experimental history of this dog (40-32) follows Table 7.

Table 10 (dog 46-22) shows a response to the deletion of histidine from the amino acid mixture which is more definite. There is much weight loss (6 per cent per week) and a negative urinary nitrogen balance. The blood protein output is large. Only 1 week of histidine replacement is tabulated as the basal diet was almost wholly refused and the experiment terminated. The sudden rise in hemoglobin is interesting and indicates a flow of protein-building material into hemoglobin rather than plasma protein.

Somewhat similar experiments in hypoproteinemia have been reported (Table 2 (2)). Deletion of histidine from the amino acid mixture does not cause decrease in plasma protein production. There was weight loss and an increase in urinary nitrogen.

Experimental History—Table 10.

Dog 46–22. Female coach. Maintained in laboratory kennels for several months under optimum conditions. Mar. 21, 1946—Blood protein depletion begun. Beginning weight 18.4 kilos, blood volume 1601 cc., plasma volume 800 cc., plasma protein level 5.3 gm. per cent. A/G ratio 1.3. Daily diet of protein-free basal biscuit 400 gm., canned carrots 150 gm., yeast 3 gm., liver extract powder 2 gm. Regular double depletion experiments.

May 31, 1946—Amino acid mixture Vuj *minus l* (+) *histidine HCl* by vein (Table 10). Daily diet of protein-free basal biscuit 350 gm., synthetic vitamin mixture 8 cc. Periods 2 to 5.

TABLE 10

Amino Acid Growth Mixture Minus Histidine—Intravenous Blood Protein Output—Basal Diet in All Periods

Period	Weight	Protein intake		Food consumption	Protein 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 40-32									
1	19.0	Basal	45	100	7.9	14.1	4.1	6.3		7.2	—
2	18.4	Amino a. − histidine	121	100	6.7	24.5	4.9	14.9		18.8	15.3
3	17.9	Amino a. − histidine	119	91	6.9	19.8	4.7	12.8		18.5	17.1
4	16.8	Amino a. − histidine	116	72	6.0	20.4	4.9	14.6		18.0	15.5
5	16.4	Amino a. − histidine	119	92	6.0	1.2	4.6	0		18.5	15.2
	2.6 = 14 per cent body weight loss	Totals........			(33)	65.9	(45)	42.3	16	73.8	63.1
6	17.2	Egg	128	100	5.7	1.2	5.1	0		20.5	10.0
7	17.0	Egg	128	100	6.1	33.5	5.3	21.8		20.5	9.0
8	16.6	Egg	128	100	6.1	10.6	4.7	7.3		20.5	9.6
9	16.6	Egg	128	100	6.0	1.1	4.8	0		20.5	8.1
	0.2 = 1 per cent body weight gain	Totals........			(50)	46.4	(33)	29.1	16	82.0	36.7
		Dog 46-22									
1	16.4	Salmon	400	100	8.9	25.0	5.0	14.5		64.2	20.1
2	15.2	Amino a. − histidine	102	12	8.6	25.8	5.0	16.2		15.8	20.2
3	14.6	Amino a. − histidine	102	55	7.4	37.6	5.1	22.2		15.8	23.3
4	13.4	Amino a. − histidine	102	62	7.4	12.9	5.3	8.4		15.8	22.2
	3.0 = 18 per cent body weight loss	Totals.......			(49)	76.3	(47)	46.8	31	47.4	65.7
5	12.8	*Amino a. + histidine	91	11	10.4	2.2	4.6	0		14.0	19.4
	0.6 = 5 per cent body weight loss	Totals.........			(30)	2.2	(−9)	0	23		

* Five doses instead of six.

Plasma volumes 770 cc., 779 cc., 715 cc. A/G ratios 1.3, 1.3, 1.6. Period 3. Daily diet of sugar mixture 200 gm., protein-free basal biscuit 100 gm. June 21—Period 5, *l* (+) histidine HCl added to amino acid mixture. Slight diarrhea. Daily diet of protein-free basal biscuit 200 gm., synthetic vitamin mixture 8 cc. Plasma volume 638 cc. A/G ratio 1.7. Experiment terminated because of poor food consumption. Dog very quiet. Recovery on daily diet of kennel food and liver.

DISCUSSION

The *production ratio* of protein output (hemoglobin and plasma protein) to protein intake appears in all tables and invites discussion. The amino acids are tabulated as protein equivalent (Table A-method). In general one gets the impression that more blood proteins appear when one of the essential amino acids is deleted from the complete mixture—that is the ratio figure is larger. With the complete mixture of amino acids, the blood protein output is generally somewhat lower although there are many exceptions. When approximately equivalent amounts of a good protein are given under the same conditions, the blood protein output is less. The average of seventeen experiments with an essential *amino acid deleted* from the complete mixture gives a figure of 25 per cent for the production ratio—protein output to intake. The average of twelve experiments with a *complete amino acid mixture* gives a figure of 19 per cent for the production ratio. The average of eight experiments with good *diet protein* gives a figure of 15 per cent for the production ratio.

The explanation of these observations is not forthcoming but one may at least suspect that the severely depleted dog when given a whole protein (egg, lactalbumin) by mouth demonstrates a flow of protein-forming materials to organ tissues to replete these stores of protein before the expected production of new hemoglobin and plasma protein gets under way. These cells in the liver, pancreas, intestinal mucosa, spleen, and marrow normally are rich in proteins (including enzymes) which are surely in a measure depleted in these long experiments with hypoproteinemia and anemia. Sufficient depletion will presumably disturb the function of these cells—*e.g.* liver function appears to be subnormal in these dogs. It is logical therefore to expect some of the incoming diet protein to contribute to these depleted cells to increase their efficiency before the production of new hemoglobin and plasma protein is measurable. We believe the liver, gastrointestinal mucosa, spleen, and marrow cells (reticulo-endothelial system) all participate in the building of new hemoglobin and plasma protein.

It is not easy to understand the mechanism of increased blood protein production when amino acids (both the complete and incomplete mixtures) replace food protein. One may believe that body weight loss goes on with the feeding of the amino acid mixtures and that from this protein loss come materials which accelerate the production of hemoglobin and plasma protein in these depleted dogs. The amino acid mixtures (oral or parenteral) are conserved and the urinary nitrogen shows a positive balance of nitrogen. Some of this amino acid material probably goes into the new formed blood proteins. Raiding of body protein stores probably comes into this reaction as the intake of protein or amino acid decreases. Some of these questions can be answered by isotopic labeling of plasma proteins and body proteins and such experiments are now in progress.

SUMMARY

Given healthy dogs fed abundant iron and protein-free or low protein diets with sustained anemia and hypoproteinemia, we can study the capacity of these animals to produce simultaneously new hemoglobin and plasma protein.

Reserve stores of blood protein-building materials are measurably depleted and levels of 6 to 8 gm. per cent for hemoglobin and 4 to 5 gm. per cent for plasma protein can be maintained for weeks or months depending upon the intake of food proteins or amino acid mixtures. These dogs are very susceptible to infection and various poisons. Dogs tire of these diets and loss of appetite terminates many experiments.

Under these conditions (double depletion) standard growth mixtures of essential amino acids are tested to show the response in blood protein output and urinary nitrogen balance. As a part of each tabulated experiment one of the essential amino acids is deleted from the complete growth mixture to compare such response with that of the whole mixture.

Methionine, threonine, phenylalanine, and tryptophane when singly eliminated from the complete amino acid mixture do effect a sharp rise in urinary nitrogen. This loss of urinary nitrogen is corrected when the individual amino acid is replaced in the mixture.

Histidine, lysine, and valine have a moderate influence upon urinary nitrogen balance toward nitrogen conservation.

Leucine, isoleucine, and arginine have minimal or no effect upon urinary nitrogen balance when these individual amino acids are deleted from the complete growth mixture of amino acids during 3 to 4 week periods.

Tryptophane and to a less extent phenylalanine and threonine when returned to the amino acid mixture are associated with a conspicuous preponderance of plasma protein output over the hemoglobin output (Table 4).

Arginine, lysine, and histidine when returned to the amino acid mixture are associated with a large preponderance of hemoglobin output.

Various amino acid mixtures under these conditions may give a positive urinary nitrogen balance and a liberal output of blood proteins but there is always weight loss, however we may choose to explain this loss.

These experiments touch on the complex problems of parenteral nutrition, experimental and clinical.

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