AMINO ACIDS AND HEMOGLOBIN PRODUCTION IN ANEMIA*

BY G. H. WHIPPLE, M.D., AND F. S. ROBSCHEIT-ROBBINS, PH.D.

(*From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York*)

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The experiments tabulated below indicate that single amino acids as a rule are utilized in the complex reaction related to hemoglobin building under the stimulus of a continuing anemia due to blood loss. The average hemoglobin production due to an amino acid amounts to about 25 to 30 per cent that due to a standard intake of liver. Moreover it is obvious that the *optical isomers* of amino acids and related *fatty acids* are well utilized to form new hemoglobin in anemia.

When hemoglobin is formed the body must have available, iron, a pigment radicle (hemin), and a large protein molecule (globin). The dog conserves *iron* within the body with a miserly hand and takes iron into the body only when there is need and at best in these experiments can absorb 30 to 50 per cent of the iron present in the intestinal tract (6, 7). Iron intake limitation can diminish the hemoglobin production. The *pigment radicle* (hemin) can be formed readily and in considerable amounts in the anemic dog and we know of no evidence that this radicle ever limits hemoglobin production (9). *Protein* intake when limited can restrict the production of hemoglobin in anemia (8).

When amino acids (1 to 5 gm. daily) are given with the basal ration it may safely be assumed that they are practically all absorbed, along with the melange of amino acids coming from digestion of the diet protein. It is surprising that practically any amino acid fed during a 2-week period may supplement effectively the digestion products and result in a new formation of 15 to 40 gm. hemoglobin above the control base line. The body is able to supply the other needed amino acids from the diet protein, from protein stores, or from protein wear and tear. When a feeding experiment with an amino acid is frankly negative we assume that some one or more of the needed supplements were not available at the time or that the amino acid was used to fill some more urgent demand within the body.

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Experiments in dogs with hypoproteinemia (12) show that certain amino acids are of prime importance in the production of new *plasma protein*—for example cystine. We had hoped that systematic testing of amino acids would show that some one or more amino acids were of primary importance for the building of globin but this hope has not been realized. Other methods of approach however may yield information on this point.

Workers in this field dream of the perfect experiment—removal of all variables with constant results down to the last milligram. Such illusions are perhaps helpful stimuli for the investigator but as a rule in physiology we must gain knowledge by imperfect experiments and indirect approach. Simplification of diet proteins is desirable but few proteins are analytically completely understood and the body apparently can break down amino acids and from the fragments make other amino acids (18), therefore no protein diet can be called a simple diet. Even when the protein intake is reduced to zero the fasting anemic dog can make considerable new hemoglobin (40 to 50 gm.) which must come from protein stores and protein wastage (2) and this must be a complex reaction viewed from any angle.

When the rat is used the diet may be an amino acid mixture but the degradation of amino acids and reconstruction of others presumably goes on. Moreover in anemia experiments with the rat there are inevitably many inaccuracies related to the sustained anemia level, the removal of hemoglobin, and blood volume changes. Protein exchange between the body reserve protein stores, plasma protein, and hemoglobin goes on readily in the dog (20) and it is to be assumed that it goes on in the rat but no experimental data are available.

The effect of both tryptophane and histidine on hemoglobin formation has interested various workers since 1923. Hirasawa (10) reported rapid formation of hemoglobin in "various forms of anemia" when giving tryptophane and iron. Tryptophane or histidine without iron was inert. The work of Fontes and Thivolle (5) employing both tryptophane and histidine injections singly or in combination resulted in considerable controversy as to the value of these amino acids in blood formation. Both normal rabbits and dogs showed increases in hemoglobin and red cell values according to their claims. Histidine and tryptophane injected simultaneously they state gave marked increase in hemoglobin up to 26 per cent. Either amino acid alone gave only negligible increases according to their figures, although the authors claim considerable hemopoietic effect. In a further report utilizing rats and keeping them on a tryptophane-free diet, Fontes and Thivolle report a marked anemia. The few figures given in their publication show a hemoglobin drop of 28 to 52 per cent.

Alcock (1) in 1933 criticized the work of these French authors when he tried to repeat their experiments. Rats kept on tryptophane-free diets for 40 to 100 days did not develop an anemia. Alcock produced a nutritional anemia (milk) in rats then kept one series on a tryptophane deficient diet and to another series gave a supplement of tryptophane to the diet and observed recovery from anemia in both series independently of the presence of tryptophane.

Drabkin and Miller (3) in 1931 report the effect of various amino acids on hemoglobin formation in rats made anemic by prolonged milk diet. Glutamic acid plus 0.2 mg. of iron was very effective, a 70 mg. dose for the rat being optimum. Arginine also showed potency for hemoglobin formation. Tryptophane, sodium aspartate, and a "proline mixture" produced an initial increase in hemoglobin. This higher level was maintained in some instances but with sodium aspartate the anemia again became severe. Alanine and histidine were found to be of no value for hemoglobin regeneration. All were fed with the same amount of iron. In a subsequent publication leucine, cystine, glycine *dl*- amino valeric acid, and glutaric acid had no effect.

Elvehjem, Steenbock, and Hart suggested that the amino acids were not copper-free and that purified glutamic acid plus iron demonstrated no effect in nutritional anemia (4). Keil and Nelson in feeding rats for 4 to 6 weeks with arginine, glutamic acid, tyrosine, tryptophane, and aspartic acid observed negative results (11).

It should be obvious that the reaction to amino acids may differ widely in rabbits and in rats, in dogs without anemia or with a long continued anemia, in rats with a milk anemia, or in dogs with a simple anemia due to blood removal. Conflicting claims in the literature are often related to such differences in the experimental procedure and it is not safe to set up broad claims from one type of experiment relative to another type of experiment or to the conditions in human disease.

Methods

General method details have been adequately described in another place (21). The hemoglobin level is kept as constant as possible—about 45 per cent where 100 per cent is equivalent to 13.8 gm. hemoglobin. The standard salmon bread which gives the low iron values by analysis (about 2 mg. iron per 100 gm. as fed) contains no bran and the other ingredients as listed (21)—wheat and potato flour, canned salmon, sugar, canned tomatoes, cod liver oil, yeast, and a salt mixture. This bread is a complete diet, is palatable, and will maintain health in the face of this prolonged anemia.

The dogs are raised in the laboratory kennel—a mixed strain of white bull dog with some coach and a little terrier blood. When about 1 year of age the dogs are put in the anemia colony, depleted of their reserve hemoglobin stores, and standardized once or twice a year on the basal bread, iron, and liver diets. The dog number shows the year in which the anemia was begun and from that time on the anemia is constantly maintained at a level about one-third of the original normal; for example, dog 37-21 means that the anemia was begun in 1937, and the 21 is the serial laboratory dog number of that year.

Amino acids (pure crystalline form) were obtained from the Eastman Kodak Company or from Hoffman La Roche. Threonine was obtained from the University of Illinois through the courtesy of Drs. W. C. Rose and C. S. Marvel.

EXPERIMENTAL OBSERVATIONS

Hemoglobin as given by Schmidt (17) contains 15 amino acids but the analysis accounts for only 71 per cent of the protein. Leucine and isoleucine together account for 29 per cent, and with lysine and histidine make up 45 per cent of this protein. One would suspect that the feeding of one of these four amino acids would be more effective than any of the other amino acids contained in hemoglobin (aspartic acid 4.4 per cent, phenylalanine 4.2 per cent, tyrosine 3.2 per cent, arginine 3.1 per cent, alanine 4.2 per cent, tryptophane 1.3 per cent, proline 2.3 per cent, hydroxyproline 1.0 per cent, glutamic acid 1.7 per cent, serine 0.6 per cent, cystine 0.3 per cent).

TABLE 1

Glycine

Dog No.	Year of experiment	Glycine		Control net hemoglobin Output per 2 wks.			Basal bread Daily ration	
		Daily dose	Hemoglobin Net output per 2 wks.	Iron 40 mg. daily	Liver 300 gm. daily	Basal bread ration alone	Bread fed	Iron in bread as fed
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*
27-238	1934	0.5	27	53	—	20	300	15
27-238	1934	1	19	53	—	12	280	14
30-121	1934	1	47	49	82	18	450	23
34-3	1939	1	0	78	77	4	300	6
30-116	1934	2	20	42	91	18	400	20
37-84	1938	2	15	28	64	4	350	11
27-238	1934	3	12	53	—	12	300	15
37-82	1939	3	10	35	68	4	450	9
27-238	1935	3	34	38	—	20	315	16
30-121	1934	5	20	49	82	16	450	23
30-116	1934	5	32	42	91	18	400	20
29-326	1936	5	34	46	82	20	400	20

Histidine (Table 6) is a little more effective than leucine and isoleucine (Table 3) in promoting hemoglobin regeneration but no more effective than glutamic acid (Table 4) which has only 1.7 per cent representation in the protein hemoglobin.

Glycine, the simplest amino acid, can be formed in the body but is not represented in the hemoglobin analyses. One might guess that its feeding would not influence the production of hemoglobin but actually the average of the 12 experiments in Table 1 is 23 gm. of hemoglobin—about as effective as any amino acid tested. We may suppose that glycine contributes by means of combination with other amino acids or split products of amino acids (19) to form amino acids required for hemoglobin production. *Urea* has been tested in a good many experiments, so far with negative results. Table 1 also indicates that an increase in the intake of glycine above 1 gm. daily does not increase hemoglobin production.

It will be noted (Tables 1 and 2) that the *iron content* of the standard bread varies from a daily iron intake of 5 or 6 mg. to a maximum of 30 or 40 mg. The bread base line output of hemoglobin per 2 weeks varies somewhat in proportion to this iron intake but different dogs vary somewhat in their capacity to use the food iron and food factors. The output due to the given amino acid is always the *net total hemoglobin* produced over and above the basal output related to the standard basal ration. It is possible to reduce hemoglobin production to zero by limited diets very low in iron in spite of reasonable amounts of diet protein (7). Such experiments obviously would give no information about the utilization of amino acids added to this low iron diet. We believe this variation in the intake of iron in these experiments is advantageous and indicates that iron is not a limiting factor in these tabulated experiments.

Alanine (Table 2) is represented in hemoglobin (4.2 per cent) but *valine* is not listed in analyses of hemoglobin. The influence of these two amino acids on hemoglobin regeneration is not conspicuous but definite—about 14 or 15 gm. above the basal output. It is to be noted that *dl*-alanine was inert in 2 experiments, one with a low iron containing bread and again with a high iron bread. Valine (Table 2) is a little more potent in the *d*- form than in the *l*- form. Some of these experiments were published elsewhere (15).

Isovaleric acid (Table 2) appears to be used a little more effectively than valine, and the average of 5 experiments is 21 gm. hemoglobin. The β-hydroxy butyric acid of which threonine is an α-amino derivative, is well used and the hemoglobin output is large—30 gm. hemoglobin average of 5 experiments. We obtained threonine sufficient for a single test (Table 2) and the reaction is definite with a net output of 29 gm. of hemoglobin. It is not known whether threonine is represented in the hemoglobin molecule.

Leucine and isoleucine (Table 3) are abundant in hemoglobin (29 per cent) and would be expected to give a maximal response when added to the diet. The 12 experiments with isoleucine give an average of 17 gm. hemoglobin and the 10 experiments with leucine an average of 20 gm. hemoglobin above the basal output. It is noted that the natural forms react in the same manner as do the optical isomers and doubling the intake (*dl*- form) does not increase hemoglobin output.

Glutamic acid (Table 4) is an important amino acid (19) and Table 4

TABLE 2

Alanine, Valine, Isovaleric Acid, Threonine, and β-Hydroxybutyric Ester

Dog No.	Year of experiment	Alanine d- natural		Control net hemoglobin Output per 2 wks.			Basal bread Daily ration	
		Daily dose	Hemoglobin Net output per 2 wks.	Iron 40 mg. daily	Liver 300 gm. daily	Basal bread ration alone	Bread fed	Iron in bread as fed
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*
33-13	1937	1	41	46	103	36	400	42
33-14	1938	1	25	58	72	4	325	10
29-326	1938	1	2	57	84	4	275	9
35-4	1937	1	20	48	89	8	500	24
		Alanine dl- synthetic						
30-116	1935	1	25	42	92	20	425	22
26-102	1934	1	24	50	76	10	225	11
33-14	1938	1	0	58	72	4	240	8
29-326	1938	1	2	56	94	4	300	10
35-4	1939	1	0	56	89	50	450	35
		Valine d- natural						
35-7	1937	1	21	55	86	22	350	21
32-5	1937	1	3	69	86	34	290	30
29-326	1938	1	4	57	84	4	300	9
35-4	1939	1	26	48	89	4	330	6
35-2	1939	1	35	48	104	4	420	8
		Valine l- isomer						
36-11	1938	1	12	65	66	4	450	14
33-14	1938	1	7	58	72	4	325	10
35-7	1937	1	21	51	82	4	380	18
32-5	1939	1	3	58	98	4	450	9
32-5	1939	1	17	58	98	60	375	27
35-6	1939	1	0	52	64	44	450	30
		Isovaleric acid						
34-148	1938	1	11	52	85	4	450	11
35-2	1938	1	10	48	104	4	350	9
35-7	1938	1	32	51	82	4	340	9
35-4	1938	1	48	48	89	12	500	13
32-5	1939	1	7	58	98	64	350	27
		Threonine dl- synthetic						
35-7	1939	1	29	51	100	4	270	5
		β-hydroxybutyric ester						
37-21	1939	1	34	72	107	4	450	9
33-14	1939	1	47	58	89	4	325	6
35-7	1939	1	46	51	100	4	250	5
34-149	1939	1	17	57	96	4	450	9
34-145	1939	2	8	53	102	22	400	8

shows that it is well utilized to build hemoglobin—an average of 25 gm. hemoglobin net output in 8 experiments. Glutamic acid is no more effective in larger doses (3 to 5 gm. daily) than in the usual 1 to 2 gm. doses, and the *iron content* of the bread does not influence the *net output* related to the amino acid. *Glutaric acid* (the fatty acid related to glutamic acid)

TABLE 3

Isoleucine and Leucine

Dog No.	Year of experiment	Isoleucine *d*- natural		Control net hemoglobin Output per 2 wks.			Basal bread Daily ration	
		Daily dose	Hemoglobin Net output per 2 wks.	Iron 40 mg. daily	Liver 300 gm. daily	Basal bread ration alone	Bread fed	Iron in bread as fed
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*
35-7	1938	1	23	51	89	4	336	11
35-4	1939	1	20	48	89	4	450	9
29-326	1939	1	0	56	94	3	310	6
		Isoleucine *l*- isomer						
32-3	1938	1	2	42	79	4	400	10
34-148	1938	1	7	66	92	4	450	14
35-4	1939	1	14	48	89	4	450	9
35-2	1939	1	26	48	104	4	450	9
29-326	1939	1	18	56	94	36	250	21
		Isoleucine *dl*- synthetic						
35-7	1939	2	19	51	82	4	350	7
35-2	1939	2	14	48	104	14	410	8
35-6	1939	2	17	52	64	44	450	30
35-4	1939	2	48	48	89	50	450	35
		Leucine *l*- natural						
27-236	1934	1	30	57	86	18	375	19
26-102	1935	1	12	39	84	6	200	10
33-14	1939	1	14	58	89	4	340	7
26-102	1934	2	27	50	76	10	225	12
29-326	1936	3	16	46	70	12	340	17
		Leucine *d*- isomer						
29-326	1937	1	21	57	107	16	250	15
33-13	1937	1	31	43	103	34	400	42
30-121	1937	1	18	54	91	24	450	27
35-7	1939	1	28	51	82	4	380	7
33-14	1939	1	9	58	89	4	350	7

in a single experiment shows a positive reaction—20 gm. hemoglobin net output—corresponding to the glutamic acid reaction.

Aspartic acid (Table 4) is well represented in hemoglobin (4.4 per cent) and gives an average response—25 gm. hemoglobin net output in 4 experi-

TABLE 4

Glutamic Acid, Glutaric Acid, Aspartic Acid, and Asparagine

Dog No.	Year of experiment	Glutamic acid *d*- natural		Control net hemoglobin Output per 2 wks.			Basal bread Daily ration	
		Daily dose	Hemoglobin Net output per 2 wks.	Iron 40 mg. daily	Liver 300 gm. daily	Basal bread ration alone	Bread fed	Iron in bread as fed
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*
27-238	1934	1	52	53	—	20	362	18
29-326	1934	2	10	33	74	12	315	16
34-149	1939	2	32	57	96	4	450	9
27-238	1934	3	26	53	—	16	375	19
27-238	1934	3	30	51	—	24	200	10
24-26	1932	5	9	63	70	4	250	13
30-121	1936	5	27	54	98	24	450	23
32-5	1939	5	14	58	98	4	350	7
		Glutaric acid						
34-149	1939	1	20	57	96	4	450	9
		Aspartic acid *l*- natural						
29-326	1934	1	19	52	68	12	300	15
30-116	1936	1	21	42	92	20	425	22
36-11	1938	1	20	58	66	4	450	14
37-21	1939	1	38	72	107	4	450	9
		l- Asparagine						
35-2	1937	1	66	42	101	34	400	42
29-326	1937	1	21	57	107	16	265	28
36-11	1938	1	0	58	66	4	450	14

ments. Asparagine gives a very irregular response. It is probable that asparagine in the intestine would react like aspartic acid.

The sulfur containing amino acids (Table 5) are of particular interest because cystine is of prime importance in the regeneration of plasma protein in dogs (12). It is noted in Table 5 that cystine does give a substantial response—regeneration of 25 gm. hemoglobin above the base line control—an average of 5 experiments. There is no evidence that the dog can use 2 gm. of cystine more effectively for hemoglobin production than the standard 1 gm. dose.

TABLE 5

Cystine and Methionine

Dog No.	Year of experiment	Cystine *l*- natural		Control net hemoglobin Output per 2 wks.			Basal bread Daily ration	
		Daily dose	Hemoglobin Net output per 2 wks.	Iron 40 mg. daily	Liver 300 gm. daily	Basal bread ration alone	Bread fed	Iron in bread as fed
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*
30-116	1934	1	44	42	91	18	400	20
37-82	1938	1	27	50	76	4	450	14
33-14	1939	1	16	58	89	4	285	5
29-326	1934	2	29	52	81	4	300	15
29-326	1935	2	11	33	74	16	350	18
		Methionine *dl*- synthetic						
26-102	1935	1	3	39	84	16	210	11
35-7	1937	1	3	49	89	24	300	32
35-6	1939	1	0	42	67	44	450	30
34-149	1938	1	18	57	96	20	450	14
34-149	1939	2	37	57	96	4	450	9
35-7	1939	2	64	51	100	4	325	6

TABLE 6

Histidine

Dog No.	Year of experiment	Histidine *l*- natural		Control net hemoglobin Output per 2 wks.			Basal bread Daily ration	
		Daily dose	Hemoglobin Net output per 2 wks.	Iron 40 mg. daily	Liver 300 gm. daily	Basal bread ration alone	Bread fed	Iron in bread as fed
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*
29-68	1932	1(v)	28	67	95	14	225	12
34-148	1939	1	24	66	92	4	450	9
27-236	1933	1	29	57	86	10	300	15
27-236	1931	1(v)	34	57	86	24	425	22
23-1	1933	1	34	46	107	14	325	17
35-6	1939	1	2	52	67	4	450	9
37-82	1939	1	18	35	68	4	450	9
35-4	1939	1	36	48	89	44	450	35
27-238	1937	1.2	34	59	—	24	300	32
29-326	1936	2	28	33	70	12	350	18
30-121	1936	2	0	54	87	30	450	23
		Histidine *d*- isomer						
27-238	1937	1	23	51	—	24	265	28
35-7	1937	1	22	55	86	24	300	32
35-7	1939	1	15	51	82	4	350	7
33-13	1937	1	31	46	103	30	400	42

Methionine (Table 5) is used a little less effectively than cystine and the average of 6 experiments is 21 gm. of hemoglobin net output. The utilization of methionine is less uniform than cystine and one cannot safely discuss the large outputs noted in the 2 experiments in which 2 gm. methionine daily were fed.

Histidine (Table 6) is of about the same potency as cystine. It is well represented in the hemoglobin molecule (7.6 per cent). Both monohydrochloride and dihydrochloride salts of histidine, the natural and isomeric forms, were used and no differences noted. Some of these experiments have been reported elsewhere (22). The net hemoglobin production of all 15 experiments averages 24 gm. Intravenous experiments (marked (v) in Table 6) were done early in this study and were soon discontinued for the simpler and more satisfactory feeding experiments.

TABLE 7

Lysine and Arginine

Dog No.	Year of experiment	Lysine *d*- natural		Control net hemoglobin Output per 2 wks.			Basal bread Daily ration	
		Daily dose	Hemoglobin Net output per 2 wks.	Iron 40 mg. daily	Liver 300 gm. daily	Basal bread ration alone	Bread fed	Iron in bread as fed
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*
30-121	1934	1	36	45	82	18	450	23
37-82	1938	1	13	50	76	4	450	14
29-326	1939	1	0	56	94	34	300	23
		Lysine *dl*- synthetic						
36-11	1938	1	7	57	105	4	450	14
32-5	1938	1	25	58	91	4	350	11
35-7	1939	1	57	51	100	4	375	7
29-326	1939	1	17	56	94	34	300	23
26-102	1935	1	12	39	84	18	210	11
		Arginine *d*- natural						
30-116	1934	1	30	42	91	18	400	20
26-102	1935	1	0	39	84	10	200	10
29-326	1938	1	12	57	107	12	275	9
37-82	1939	1	9	35	68	4	450	9

Lysine and arginine (Table 7) are not particularly effective in promoting hemoglobin regeneration. They both show wide variations and negative reactions. The net hemoglobin output is 21 gm. for lysine—average of 8 experiments—and 13 gm. for arginine—average for 4 experiments. Wide variation in the iron content of the bread does not enter into the net response nor limit the reaction.

Phenylalanine (Table 8) is represented in the hemoglobin molecule (4.2 per cent). We have used both the natural, isomeric and *dl*- synthetic forms. Some of these experiments have been reported elsewhere (22). All experiments show a positive result and the reaction is fairly uniform. The

TABLE 8

Phenylalanine

Dog No.	Year of experiment	Phenylalanine *l*- natural		Control net hemoglobin Output per 2 wks.			Basal bread Daily ration	
		Daily dose	Hemoglobin Net output per 2 wks.	Iron 40 mg. daily	Liver 300 gm. daily	Basal bread ration alone	Bread fed	Iron in bread as fed
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*
29-326	1937	1	18	46	82	12	315	19
30-121	1937	1	35	54	98	28	450	27
33-13	1938	1	34	46	85	14	400	13
37-82	1939	1	11	35	60	4	450	9
		Phenylalanine *d*- isomer						
29-326	1937	1	27	46	82	12	225	14
30-121	1937	1	41	54	91	28	450	27
34-149	1938	1	18	54	99	11	450	14
33-14	1939	1	9	58	89	4	350	7
		Phenylalanine *dl*- synthetic						
26-102	1934	0.5	12	39	84	14	200	10
26-102	1934	1	25	39	76	10	255	13
30-116	1933	1	51	42	91	14	375	21
23-1	1934	1	8	67	81	16	300	15
29-326	1937	1	32	46	82	12	225	14
34-3	1939	1	7	78	77	4	315	6
23-1	1934	2	11	67	81	16	300	15
27-238	1935	3	29	63	—	24	300	15

average net output in 16 experiments is 23 gm. hemoglobin. The dog cannot use effectively more than 1 gm. daily. A repeat experiment in dog 26-102 indicates that 0.5 gm. daily is not as effective as the usual 1 gm. dose of the amino acid.

Tryptophane (Table 9) shows nothing out of the ordinary and appears to behave like the other amino acids—an average of 20 gm. hemoglobin for 5 experiments. *Tyrosine* (Table 9) in 1 gm. doses is less effective than phenylalanine (Table 8)—an average of 15 gm. net hemoglobin output in

TABLE 9

Tryptophane and Tyrosine

Dog No.	Year of experiment	Tryptophane l- natural		Control net hemoglobin Output per 2 wks.			Basal bread Daily ration	
		Daily dose	Hemoglobin Net output per 2 wks.	Iron 40 mg. daily	Liver 300 gm. daily	Basal bread ration alone	Bread fed	Iron in bread as fed
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*
26-164	1934	1	27	46	80	10	270	14
27-238	1935	1	24	63	—	24	350	18
29-326	1937	1	21	46	82	12	250	15
33-14	1939	1	11	58	89	4	325	6
37-84	1939	1	16	68	76	4	450	4
		Tryosine l- natural						
29-326	1935	1	10	33	74	16	310	16
33-14	1938	1	11	58	72	10	283	9
30-121	1938	1	5	54	73	4	450	14
35-7	1939	1	19	51	100	4	400	8
27-236	1934	1	30	57	86	18	315	16
27-236	1934	2	39	57	86	20	350	18
26-164	1934	2	38	46	80	12	275	14

TABLE 10

Proline and Hydroxyproline

Dog No.	Year of experiment	Proline l- natural		Control net hemoglobin Output per 2 wks.			Basal bread Daily ration	
		Daily dose	Hemoglobin Net ouput per 2 wks.	Iron 40 mg. daily	Liver 300 gm. daily	Basal bread ration alone	Bread fed	Iron in bread as fed
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*
27-236	1932	1	35	57	77	10	400	20
27-238	1937	1	43	51	—	30	235	25
33-14	1939	1	34	58	89	4	300	6
34-148	1939	1	25	66	92	4	450	9
37-21	1939	1	32	72	107	4	450	9
27-238	1931	1(v)	17	48	—	14	280	14
		Hydroxyproline l- natural						
29-326	1934	1	7	52	81	4	300	15
33-14	1937	1	13	58	72	20	280	17

5 experiments, but 2 gm. doses seem to be more effective—38 and 39 gm. hemoglobin.

Proline and hydroxyproline (Table 10) differ in their potency for hemoglobin regeneration. We could not obtain enough hydroxyproline to give a fair analysis but the 2 experiments cited show a low net hemoglobin output. *Proline* in contrast gives rather high values—31 gm. net hemoglobin output, an average of 6 experiments. One intravenous experiment (v) in Table 10 shows a rather low output of hemoglobin and perhaps should not be included in the average.

DISCUSSION

During the past eight years as occasion and materials permitted we have been testing individual amino acids in well standardized anemic dogs to see how much new hemoglobin would be produced over and above the control base line output. Various doses have been given but 1 gm. per day seems to be the optimum intake for most of the amino acids for a 2-week period. Larger doses rarely increase the output of hemoglobin but smaller doses in some experiments do decrease the output.

Hemoglobin contains 15 amino acids as listed by Schmidt (17) but this analysis accounts for only 71 per cent of the protein. Of these amino acids 7 are listed by Rose (16) as essential for growth—leucine and isoleucine, histidine, lysine, phenylalanine, tryptophane, and arginine. These essential amino acids are no more potent than the others when measured by their capacity to promote hemoglobin production in this type of anemia due to blood loss.

Hemoglobin is a peculiar basic protein and it is possible that its fabrication within the body differs in many respects from that of plasma proteins and cell proteins. Its disintegration also may be different and we know very little about what happens to the globin fraction when the red cells wear out or are destroyed. The plasma depleted dog cannot use globin to make new plasma protein (12) nor is this globin conserved for the protein needs of the body in fasting experiments except to produce new hemoglobin (13). In contrast the plasma protein can supply the protein needs of the fasting dog.

Globin can contribute to the building of hemoglobin (14) in the standard anemic dog and as horse globin can be utilized it seems safe to assume that this globin is broken down somewhat before contributing to the upbuilding of dog hemoglobin. Globin fed by mouth is very well utilized to form hemoglobin, an average return of 30 to 40 gm. hemoglobin from the feeding of 100 gm. globin—to be compared with standard liver protein feeding, an average return of 13 gm. hemoglobin from the feeding of 100 gm. liver protein.

SUMMARY

Certain individual amino acids when given to standard anemic dogs cause an increase in new hemoglobin production. Occasional negative experiments are recorded.

Glycine, glutamic acid, aspartic acid, cystine, histidine, phenylalanine, and proline when given in 1 gm. doses daily for 2 weeks, increase hemoglobin output on the average 23 to 25 gm. above the control level. This reaction amounts to 25 to 30 per cent of the new hemoglobin produced by the feeding of 300 gm. liver daily for 2 weeks—a standard liver test.

Alanine, valine, isoleucine, and arginine in the same dosage increase the hemoglobin output on the average 13 to 17 gm. per 2 weeks over the control level.

Leucine, methionine, lysine, tryptophane, and tyrosine fall in a middle group with hemoglobin output of about 20 gm.

Isovaleric acid, β-hydroxybutyric acid, glutaric acid, and asparagine have shown positive effects and the *butyrate* is unusually potent for hemoglobin production (Table 2).

The isomeric and *dl*- synthetic forms of the amino acids are as effectively utilized in this reaction as are the natural forms.

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