Studies on Amino Acid Inadequacy of Blood.* (23009)

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Azotemia is a clinical sign of massive gastrointestinal bleeding(1-6). Several interesting studies relate digestion of large amounts of hemoglobin to increased blood NPN in the absence of shock, dehydration, starvation, or impaired renal function(1,5,7). Catabolism of large amounts of any protein may be followed by an increase in blood urea(8). Hemoglobin feeding, however, appeared to produce a proportionately greater increase in blood urea of dogs than casein and ground beef(9). Deficiencies of certain amino acids also increase blood NPN(10-12). This suggests that the known amino acid imbalance of blood(13) contributes to the azotemia. These studies further evaluate human blood and plasma and canine blood as sources of essential amino acids for protein depleted adult male rats. They also explore, in a preliminary way, the relationship of amino acid imbalance to azotemia following ingestion of large amounts of blood.

Experimental. The procedure used by Frost(13) was modified for protein repletion experiments. Young male rats of the Abbott stock weighing 150-200 g were prepared for assay by 12-day depletion on a low-protein diet (NP 14).† They were then oriented to drinking protein solution by a 3-day drink trial with a modified fibrin hydrolysate.‡ The rats were again depleted for 3 days and divided into groups. The citrated blood or plasma (Formula B, U.S.P. and N.I.H.) for each experiment was pooled. Where additions were made on a nitrogen basis, suitable aliquots were analyzed for Kjeldahl-nitrogen. Blood was then diluted with water and solutions of DL-methionine and L-isoleucine so 18 ml of final solution contained 120 mg of blood-N. When blood was not diluted, the additions were blended with the blood in a Waring blender. Enough solution was prepared to last the term of each experiment. All solutions were stored in the refrigerator. During preparatory and assay periods the rats had access to low-protein diet and deionized water. The rats were allotted 120 mg N/rat/day unless otherwise stated. The volume of solution taken daily by each rat was recorded and the rats were weighed at suitable intervals about 20 hours after the preceding feeding. Group variations were expressed as standard error (S. E. $= \sqrt{Sx^2/n(n-1)}$). In the rat experiments on blood urea-N 300 g males of Abbott stock were fasted 17 hours. Then citrated human blood or blood supplemented with 1.15 g of L-isoleucine and 0.31 g of DL-methionine/100 ml was administered by gastric intubation under light ether anesthesia. The rats which ingested about 24 ml of blood received 2 doses with one hour elapsing between initial 14 ml dose and final 10 ml dose. Time was counted after initial intubation. The rats receiving about 14 ml of blood were fed only once. Similar fasted and anesthetized rats were the controls. One ml oxalated blood samples were obtained by heart puncture for urea-N determinations(14) and normal feeding was not allowed until one sample was removed from each rat. A similar experiment employed groups of 2 dogs each. The dogs fasted for 2 days and then received lyophilized dog blood and citrated human blood at daily intakes of 300 and 375 mg N/kg body weight respectively for 11 days. A non-

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† NP 14: Sucrose, 85%; Jones and Foster Salt Mixture (28), 4; Agar, 1.4; hydrogenated vegetable oil, 4; corn oil, 4; Dry vit. A and vit. D mixture (8000 I. U. vit. A and 1500 I. U. vit. D/g) .1; choline chloride, .1. Vitamins, mg/100 g of diet: thiamine hydrochloride, .6; Ca DL-pantothenate, 5; inositol, 5; folic acid, .3; menadione sodium bisulfite, .1; vit. B_{12} concentrate (3 mg vit. B_{12}/g) .1.

‡ Aminosol (trade mark), 5%, Abbott Labs., N. Chicago.

TABLE I. Protein Repletion by Human Blood and Plasma (120 mg N/Rat/Day; 6 Rats/Group).

N source	Route	Supplement, % (W/V) Iso-leucine	Methio-nine	Avg 10-day gain, g ± S.E.	% allotment consumed
Blood	Tube			− 9 ± 2.02	100
	"	.7	.45	14 ± 2.6	100
	Oral			−18 ± 2.7	18
	"	.7	.45	30 ± 1.4	100
Plasma	Oral			11 ± 2.5	77
	"	.32		31 ± 1.9	100
	"	.32	.19	41 ± 1.7	100
Fibrin hydrolysate	Oral			33 ± 2.0	100

TABLE II. Isoleucine and Methionine Deficit in Blood (Protein Repletion: 5-9 Rats/Group).

Exp.	Source of blood	Supplement, mg/120 mg N Iso-leucine	Methio-nine	Avg 10-day gain, g ± S.E.	% allotment consumed
I	Canine			−11 ± 1.1	21
			30.4	−14 ± 1.4	20
		41.6		− 2.4 ± 1.4	57
		41.6	30.4	33 ± 1.3	100
I	Human			−16 ± 1.0	10
			20.3	−14 ± 2.0	10
		43.7		11 ± .9	100
		43.7	20.3	22 ± 1.7	100
II	Human	22	20.3	− 3 ± 1.9	85
		33	"	7.5 ± 3.3	89
		44	"	16 ± 1.8	100
		55	"	20 ± 1.4	97
		"	10	13.3 ± 1.1	100
		"	15	17.3 ± 1.4	100
		"	25	17.7 ± 1.8	100
		"	30	21.8 ± 1.4	100

protein diet mixture (NP 13X)§ supplied the other known nutrients and 70 calories/kg body weight/day. Blood and non-protein diet were fed at 9 a. m., 1 p. m., and 3 p. m. Blood samples were taken daily at 8:45 a. m. and on the eleventh day an additional sample was taken at 4 p. m. Blood samples were analyzed for urea(15) and NPN(16) and nitrogen balance of the dogs was followed. After 11 days the dogs were fed 150 mg N/kg/day as beef fibrin for 3 days and were then given 150 mg N/kg/day as human blood for 4 days. The blood and non-protein diet were fed at 9 a. m. and 3 p. m. and blood urea and NPN and nitrogen balance were followed as before. Amino acid analyses were performed on hydrolyzed human blood and plasma and dog blood. Microbiological assays(17,18,19) were performed after refluxing with excess 5 N hydrochloric acid for 5-6 hours. Samples for chemical tryptophan analysis(20) were refluxed with 5 N sodium hydroxide for 5 hours. Additional samples were analyzed for Kjeldahl-nitrogen and all analyses were calculated as grams of amino acid/16.0 g N of the original materials. Three samples of human blood, and 2 samples of human plasma and dog blood were analyzed and the results averaged.

§ NP 13X: Sucrose 69.4%; hydrogenated vegetable oil 18; corn oil 3; Oladol (U.S.P. units/g: 55,000 A and 5500 D), .5; salt mixture(28) 4; agar 2.50; choline chloride .30; wheatgerm oil .25; liver fraction "O" .2. Vitamins, mg/100 lb of diet: inositol 2270; p-aminobenzoic acid 45; thiamine hydrochloride 272; riboflavin 272; nicotinamide 5450; pyridoxine hydrocloride 182; Ca DL-pantothenate 917; folic acid 50.

TABLE III. Rats' Blood Urea-N after Ingestion of Human Blood.

Supplement, % (W/V)			Avg urea-N, mg %, after		
Iso-leucine	Methio-nine	ml/rat	3 hr	5 hr	7 hr
		24 (4)	24 (1)	26 (1)	29 (2)
		14 (6)	20 (2)	27 (2)	26 (2)
1.15	.31	23 (4)	22 (1)	30 (1)	27 (2)
1.15	.31	14 (6)	22 (2)	24 (2)	22 (2)
		0(10)	15 (3)	13 (3)	15 (4)

No. of rats in parentheses.

Results. The results of these experiments confirm and extend the earlier report on inadequacy of human blood for the protein depleted adult male rat(13). Human blood and plasma and canine blood are deficient in both isoleucine and methionine when used as protein sources for depleted rats (Tables I and II). When fed to supply 120 mg N/rat/day, dog blood was as deficient in isoleucine as human blood and somewhat more deficient in methionine. Approximately 55 mg of L-isoleucine and 15 mg of DL-methionine/120 mg of blood-N adequately supplemented human blood for protein repletion in the rat (Table III). Using the average L-isoleucine and L-methionine content of human blood obtained by analyzing 3 different samples (Table IV), these figures approximate the reported repletion minima(22). As previously reported(13), the rats refused the non-supplemented blood after the first few days of repletion. As expected, nutritive value of human plasma was enhanced somewhat by addition of methionine and isoleucine(21). The improvement, however, was not as great as in the case of whole blood. Plasma also proved more readily acceptable than blood, confirming its better amino acid balance.

TABLE IV. Approximate Amino Acid Content of Blood, Plasma, and Fibrin Hydrolysate. (g/16.0 g N.)

Amino acids	Human blood	Dog blood	Human plasma	Fibrin hydrol-ysate
Isoleucine	.80	1.49	2.26	6.15
Leucine	15.4	18.1	11.95	15.3
Valine	9.51	9.12	7.86	6.4
Threonine	4.86	5.36	4.01	6.4
Methionine	1.24	.59	2.30	3.20
Phenylalanine	6.84	7.05	6.21	3.84
Histidine	6.42	5.19	3.21	2.44
Arginine	3.60	3.15	4.43	6.00
Tryptophan	1.34	1.06	1.61	1.23
Glutamic acid	9.13	8.25	14.47	5.16
Aspartic acid	5.40	5.57	5.22	2.36
Tyrosine	3.66	3.99	3.48	3.20
Lysine	9.20	9.76	10.11	9.1

Ingestion of 12-25 ml of citrated human blood increased blood urea-N in rats (Table IV). This agrees with the work of others with other species and other proteins(7,8,23). Supplementing the blood with 1.15 g of L-isoleucine and 0.31 g of DL-methionine/100 ml, however, did not reduce the rats' blood urea. Feeding dogs 300 mg N/kg/day as dog blood, or 150-375 mg N/kg/day as human blood, did not produce increased blood urea or NPN levels or negative nitrogen balance. Thus it would appear, either that the marked azotemia following ingestion of large amounts of blood is not related to amino acid imbalance, or the conditions in this experiment were not suited to demonstrate such an effect. When blood fortified with amino acids is given rapidly by stomach tube, the supplemental amino acids may be absorbed faster than those derived from the blood. This probably reduces the efficacy of the supplements. The rats fed supplemented blood twice daily by stomach tube (Table I), gained less than those allowed to drink the fortified blood. This suggests that the isoleucine and methionine might be more effective if given throughout the entire time interval in which the blood proteins are digested.

Analysis of blood and plasma for 13 amino acids revealed that human and dog blood differ principally in their content of isoleucine, leucine, and methionine (Table IV). Approximate amino acid content of the modified fibrin hydrolysate used is shown for comparison. Human blood contains more methionine, but less isoleucine and leucine than dog blood. These differences may be due chiefly to differences in amino acid composition of the hemoglobins(24). It is of interest that although dog blood contains twice as much isoleucine as human blood and two-thirds as much as human plasma, supplementing it with methionine did not increase the gains of rats (Table I). The excess of leucine in dog

blood may increase the isoleucine requirement. Thus, it has been shown that leucine increases the rat's isoleucine requirement(25) and that infusion of leucine into the blood of dogs increases urinary excretion of isoleucine as much as 140 times(26). Excess leucine reduced the rate of intestinal absorption of isoleucine by about 25%(27).

Summary. (1) These studies further evaluate whole blood as sources of essential amino acids for protein depleted adult male rats. Human and canine blood again were rejected and failed to support repletion. Human blood supported rapid repletion when supplemented with 55 mg L-isoleucine and 15 mg DL-methionine per 120 mg of blood-N. Less than half these levels added to plasma supported repletion. Plasma alone permitted an intermediate rate of repletion. (2) Feeding normal dogs a non-protein diet and 150-375 mg N/kg/day as lyophilized human or dog blood did not increase the blood urea-N or NPN or produce negative N balance. Force-feeding 12-25 ml of citrated human blood increased blood urea-N of fasting rats. Supplementing the blood with isoleucine and methionine, however, did not reduce the blood urea-N. (3) Analysis of human blood, human plasma, and dog blood for 13 amino acids is reported.

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