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Research Article

STUDIES ON AMINO ACID METABOLISM. II. BLOOD GLYCINE AND TOTAL AMINO ACIDS IN VARIOUS PATHOLOGICAL CONDITIONS, WITH OBSERVATIONS ON THE EFFECTS OF INTRAVENOUSLY ADMINISTERED GLYCINE[1]

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INTRODUCTION

Glycine plays a role in the metabolism of serine (1), glutamic acid (2), glyoxylic acid (3), uric acid (4–6), lactate (6), creatine (7), glutathione (8), and the porphyrin of hemoglobin (9, 10). The amino acid has been administered, with inconclusive results, to patients with myasthenia gravis (11, 12), and with peripheral vascular disorders because of its vasodilating effects (13). Moreover, knowledge of the life span of erythrocytes (14) and of red cell destruction (15) has been advanced by using tagged glycine.

The normal concentration of glycine in blood, plasma, and erythrocytes has been reported (16) but nothing is known regarding its level in pathological conditions. The present data consist of glycine and total α amino N levels in patients with various diseases and following infusions of the amino acid.

METHODS

Glycine and total α amino N were measured by techniques previously described (17, 18). Each value is the average of duplicate determinations.

For the glycine tolerance tests, a sterile 10 per cent aqueous glycine solution (Eastman) was injected at 30 cc. per minute (total amount—1 gm. of the amino acid per 15 lbs. of body weight). In three subjects less glycine was administered. Blood glycine was determined before, and at intervals after, the injection. The urinary excretion of glycine during the experiments was also measured. Food was withheld for 12 hours prior to, and throughout, the test, but water was permitted *ad libitum*.

RESULTS

Normals: Based on previous data (16) and a few additional observations on normal subjects, the normal glycine range is now considered to be: for blood, 1.76–3.02 mgm. % (mean 2.10; S. D. 0.26); for plasma, 1.47–2.83 (mean 1.87; S. D. 0.34); for red cells, 1.64–3.73 (mean 2.49; S. D. 0.51). Any value for glycine or α amino N which deviated from the normal mean by an amount exceeding twice the standard deviation was considered significantly abnormal.[3]

Diseases of the liver and bile ducts: Five of 11 patients with liver disease had jaundice of hepatocellular origin; in two others the jaundice was either obstructive or hepatocellular. Subnormal blood and plasma glycine were found in one subject (subacute yellow atrophy [Table I, No. 10]) while in another (acute infectious hepatitis [No. 14]) plasma glycine was low whereas whole blood glycine was normal. Erythrocyte glycine was significantly elevated in five, contributing in four to a high whole blood glycine. These individuals had definite evidence of parenchymatous involvement.

Case P. L. with severe liver damage (Table I, Nos. 10–13) is of particular interest. Although determinations were not done until high protein feeding by stomach tube had already been initiated (with clinical improvement), blood and plasma glycine and total α amino N values were extremely low. As the patient continued to improve, plasma glycine and blood α amino N returned to normal while red cell glycine became abnormally high.

In two cases of liver disease, an intravenous glycine tolerance test was essentially normal.

Elevated red cell amino N was observed in three patients in one of whom plasma α amino N was also increased. No correlation between glycine, α amino N and protein concentration was evident.

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[3] In order to conserve space, only subjects who showed abnormal glycine values are recorded in Tables I and II. Should the reader desire additional data he may communicate with the authors.

Thus, except for low glycine and amino N in one case of subacute yellow atrophy, returning to normal with clinical improvement, no remarkable findings in plasma glycine were observed. Noticeable, however, was the tendency to elevated glycine and α amino N in the red cells.

Diseases of the kidney and essential hypertension: In two patients with acute glomerulonephritis the glycine values were normal, despite azotemia. Of nine subjects with chronic glomerulonephritis and renal insufficiency glycine concentrations were significantly increased in six and appeared unre-

TABLE I

Blood glycine and α amino N *in various diseases*

No.	Subject	Date		Amino N			Glycine			Ict. ind.	Total prot.	Alb.	Glob.
				Blood	Plasma	Erythrocytes	Blood	Plasma	Erythrocytes				
			Diseases of the liver and bile ducts										
				mgm. %	*mgm. %*	*mgm. %*	*mgm. %*	*mgm. %*	*mgm. %*		*gm. %*	*gm. %*	*gm. %*
10	P. L.	2/26/46	Sub yel. atrophy	2.63	2.69	2.50	1.64	1.17	2.63	49	5.41		
11	P. L.	3/2/46	Sub yel. atrophy	4.49	3.05	7.30	2.62	1.67	4.46	31			
13	P. L.	3/25/46	Sub yel. atrophy	4.88	3.30	7.45	3.03	2.26	4.29	15	7.07		
14	A. E.		Infect. hepatitis	5.13	3.42	10.20	2.10	1.35	4.36	17	5.90	2.45	3.54
16	L. S.	1/12/47	Infect. hepatitis	9.20	7.60	12.00	3.40	2.19	5.55	55			
18	L. S.	2/6/47	Infect. hepatitis	5.94	4.42	7.05	2.87	2.06	3.99	26			
24	J. R.		Cirrhosis; jaundice				3.07	1.98	4.43	34	6.25	2.78	3.47
28	R. H.		Chr. hepatit.; ? malignancy	3.92	2.78	6.24	2.95	2.33	4.21	6	8.00	3.32	5.08
			Renal disease and essential hypertension										
										NPN *mgm. %*			
33	N. S.		Chr. glomerul.; osteoporosis	4.52			3.56	3.64	3.25	45	6.63		
34	I. C.		Chr. glomerul.; uremia	6.03	5.14	8.58	3.08	3.04	3.19	75	6.70		
35	I. Z.		Chr. glomerul.; uremia hypert.	7.20	3.99	16.30	2.58	2.11	3.91	97	7.01		
36	J. Z.		Chr. glomerul.; uremia hypert.		3.86		2.96	2.96		166	7.01	3.99	3.02
39	M. K.		Hypert.; urem.; myocard. infarct.				2.24	1.72	3.57	150			
41	E. W.		Uremia; sulfadiazine kidney				2.48	2.95	1.34	96	5.24		
43	M. C.		Chr. glomerul.; nephrosis; uremia	4.72	3.14	10.30	3.53	3.00	5.40	50	4.04	1.82	2.22
44	J. M.		Chr. glomerul.; nephrosis; diab.				2.71	1.62	4.28	34	5.69	3.59	2.10
47	R. R.		Hyperten.; diabetes	5.02	3.86	7.09	2.69	2.31	3.36	35	6.10		
49	B. F.		Essential hypertension	5.81	4.80	7.07	2.65	2.06	3.39	37	7.89		
			Endocrine diseases										
										BMR			
51	B. K.		Hyperthyr. and myopathy; untreated				1.54			+40			
52	B. K.		Same; KI treatment				1.32	1.16	1.55	+24			
53	F. K.		Hyperthyr., KI treatment				2.59	1.71	3.57	+30	7.17		
54	M. S.		Hyperthyr. (postthyroidectomy) untreated	6.70	4.35	10.00	3.80	2.98	4.93	−25			
55	C. L.		Hypothyr.; essent. hypertension				4.50	3.57		−16	6.22		
56	E. L.	1/6/47	Cretinism, untreated	8.03	4.41	14.70	3.55	2.78	4.97	−20	7.32	4.89	2.43
57	E. L.	1/8/47	Cretinism, untreated				3.59	3.27	4.17	−25			
58	M. H.	4/14/47	Acromegaly (preoperatively)				3.56	2.89	4.45	+20	6.76		
59	M. H.	4/25/47	Acromegaly (preoperatively)				3.46	3.01	4.10	+10			
60	M. H.	4/30/47	Acromegaly (postoperatively)				3.38	3.02	4.00				
61	M. H.	5/7/47	Acromegaly (postoperatively)				3.47	2.96	4.33	+ 3			
62	M. H.	5/28/47	Acromegaly (postoperatively)				3.40	2.84					
66	M. W.		Scleroderma and Addison's dis. (salt and Doca)	6.03	3.29	10.00	3.04	2.25	4.19	+ 4	6.09	3.48	2.63
67	E. A.	11/19/46	Malnutrit.; hypoprot.; hypometab.	4.38	2.16	8.98	1.90	1.40	2.93		3.65	1.27	2.38
68	E. A.	11/21/46	Malnutrit.; hypoprot.; hypometab.	4.69	2.69		4.70	3.30					
69	E. A.	12/26/46	Malnutrit.; hypoprot.; hypometab.	7.88	3.20		5.66	3.24		−29	3.86	1.12	2.74
70	E. A.	1/2/47	Malnutrit.; hypoprot.; hypometab.					3.51			4.00	2.29	1.71
71	E. A.	1/29/47	Malnutrit.; hypoprot.; hypometab.	5.70	3.39		5.12	3.04					
72	E. A.	2/2/47	Malnutrit.; hypoprot.; hypometab.				5.91	3.86			3.72	1.71	2.01

TABLE I—*Continued*

No.	Subject	Date		Amino N			Glycine			BMR	Total prot.	Alb.	Glob.
				Blood	Plasma	Erythrocytes	Blood	Plasma	Erythrocytes				
			Diseases of muscle										
				mgm. %	*mgm. %*	*mgm. %*	*mgm. %*	*mgm. %*	*mgm. %*		*gm. %*	*gm. %*	*gm. %*
74	A. L.	4/6/46	Myasth. grav.; testost. treat.	9.48	6.37	13.40	2.62	2.29	3.04				
75	A. L.	6/25/46	Myasth. grav.; testost. treat.	8.45	6.05	11.00	3.03	2.56	3.51				
76	R. H.	12/15/45	Progress. musc. dystrophy	4.85	2.95		1.56						
77	R. H.	2/12/47	P.M.D.—after stilbest. treat.	5.55	3.36	8.81	2.61	1.83	3.75		7.38	5.08	2.30
78	R. H.	2/24/47	P.M.D.—after stilbest. treat.				2.98	2.78	3.31	−24			
79	R. H.	2/25/47	P.M.D.—after stilbest. treat.				2.91	2.06	4.27				
80	R. H.	3/11/47	P.M.D.—after stilbest. treat.				3.08	2.18	4.55				
			Diseases of skin										
81	E. G.		Pemphigus; treat. w/nirvanol	8.00	4.62	14.80	3.62	2.29	6.63		6.43		
84	A. D.		Lupus erythem. dissem.				2.50	1.67	4.11	+11	5.66	2.63	3.03
86	E. C.		Urticaria				2.69	2.17	3.56				
			Blood diseases										
89	M. G.		Polycythemia vera; treated with urethane				3.76	1.19	5.48		8.47	3.60	3.64
90	B. M.		Polycythemia vera				3.51	2.38	4.13		5.69		
91	M. K.		Myelogen. leuk. and polycyth. vera				3.36	2.14	5.20				
92	J. F.	5/2/47	Chronic myelog. leukemia				3.21	2.14	5.35	+38	6.90		
93	J. F.	5/23/47	Chronic myelog. leukemia treated with urethane				2.27	1.45					
			Miscellaneous										
94	G. F.		Rupt. interverteb. disc				2.62	2.08	3.78				
96	G. F.		Rupt. interverteb. disc				2.69	1.83	4.48				
99	O. K.		Bronchial asthma				2.79	1.98	3.68				
103	I. R.		Carc. pancreas; metast.	4.62	2.95	7.41	2.78	1.99	4.10		6.05		
106	M. Z.		Chr. ulc. colitis; malnut.				1.77	1.19	2.37		6.53		
107	A. B.		Chr. ulc. colitis; malnut.				1.56	1.61	1.41		4.32		
118	P. Y.	3/28/47	Rheumat. arthritis				1.37	1.11	1.91				
119	P. Y.	4/4/47	Rheumat. arthritis				1.65	0.96	3.27				
123	P. Y.	4/23/47	Rheumat. arthritis				1.85	1.04	3.88				
124	P. Y.	4/24/47	Rheumat. arthritis				1.98	1.10	4.05				
127	J. B.*		Rheumat. arthritis				2.86	1.72	4.80				
129	S. T.*		Rheumat. arthritis				1.69	1.15	2.67		7.34	4.06	3.28

* We are indebted to Dr. Walter Bauer and Dr. Louis K. Dahl of the Massachusetts General Hospital, Boston, for generously providing these patients for our study.

lated to the degree of nitrogen retention and to α amino N which, except in two cases, was normal. The glycine tolerance test was not remarkable in two subjects with chronic nephritis, one with marked hypoproteinemia.

Endocrine disease: Five patients with hypermetabolism were studied. In one (No. 51) who showed myopathy simulating myasthenia gravis, all the glycine values were low. In another subject with acromegaly (Nos. 58–62) the values were elevated; this persisted for at least one month after ablation of the hypophysis. Blood amino N was normal in those subjects in whom it was determined.

In five cases of hypometabolism, glycine tended to be elevated, whereas in only one, a cretin (No. 56), was the α amino N also increased. In one patient with hypometabolism and nutritional hypoproteinemia plasma amino N was originally low, then steadily rose, while plasma glycine values

TABLE II

Intravenous glycine tolerance test

No.	Init.	Sex	Diagnosis	Wt.	Glycine given	Time after glyc.	Blood glycine			Glycine excret.
							Blood	Plasma	Cells	
				lbs.	*gms.*	*min.*	*mgm. %*	*mgm. %*	*mgm. %*	*mgm.*
5	C. L.	F	Essent. hyperten. hypothyroid.	210	14	0	4.50	3.57	6.08	
						20	26.7	32.2	17.4	277
						80	12.7	12.8	12.4	
						140	9.99	9.20	11.4	
						260	7.87	7.27	8.89	
6	M. H.	F	Acromegaly (one week postoperatively)	150	9	0	3.42	2.96	4.33	614
						20	15.9	20.7	7.82	
						80	9.81	11.2	7.52	
						200	6.79	6.23	7.74	
7	E. A.	F	Nutrit. hypoprot.; hypometabolism	76	5	0		3.51		34
						20		32.6		
						80		11.1		
						140		7.49		
						200		4.99		
10	P. Y.	M	Rheumatoid arthritis	121	8	0	1.87	1.28	3.25	
						20	5.88	5.54	6.07	
						80	2.96	2.14	4.91	
						200	2.61	1.36	5.58	
11	P. Y.	M	Rheumatoid arthritis, 30 gms. glycine orally 12 hours before test	121	8	0	1.98	1.10	4.05	381
						20	6.34	6.15	6.10	
						80	3.03	2.13	5.28	
						200	2.13	1.27	4.30	

increased suddenly from low normal and remained elevated. The changes could not be related to therapy.

The glycine tolerance test of two patients with endocrine disturbances, one (Table II, No. 5) with hypothyroidism and the other (Table II, No. 6) with acromegaly and hypermetabolism, showed very high plasma and red cell glycine following injection, with an abnormally slow decline thereafter. Fasting blood glycine was also abnormally high. In Case No. 7 (hypometabolism and nutritional hypoproteinemia) plasma glycine rose markedly after injection. The subsequent decline, however, was essentially normal.

Two diabetics, both insulin treated, showed low normal plasma glycine.

Elevated whole blood and red cell glycine was observed in a case of Addison's disease with scleroderma.

Diseases of muscle: Four patients with myopathies were studied. One of two cases of myasthenia gravis showed normal blood glycine and α amino N; he was receiving prostigmine therapy. In the other individual (No. 74), who was largely bedridden, glycine and α amino N were elevated. She had been taking methyltestosterone orally for nine months. Unfortunately these subjects were not studied prior to treatment.

One subject with progressive muscular dystrophy and hypometabolism (No. 76) showed low normal amino N and glycine levels one and a half years ago. After stilbestrol therapy for several months, his blood glycine became elevated. Glycine tolerance, however, was normal.

Patient B. K. (No. 51), with myopathy and hyperthyroidism, discussed before, showed low blood and plasma glycine. An oral glycine tolerance curve, reported in a previous communication (16), was abnormally flat.

Rheumatoid arthritis: This group comprised six cases, all with active disease. One patient No. 118) had moderate anemia and spiking fever. Low plasma glycine was observed in two (Nos. 118, 129) one of whom (No. 118; Table II, Nos. 10, 11) showed remarkably abnormal glycine tolerance curves. Not only were they relatively flat (Figures 1 and 2) but also they returned practically to the pre-injection values, in contrast to the

other subjects. Furthermore, glycine administered the night before the test did not affect the pattern of response. The urinary glycine of this patient during the tolerance test was not significantly different from that of other individuals. Administration of glycine (10 gms. a day for four days orally, 30 gms. orally or 8 gms. intravenously) did not influence his creatinine or creatine excretion.

Ten minutes following the first glycine injection, the patient suddenly experienced remarkable "loosening" of the joints with painless mobility for the first time in two weeks. After eight hours, the pain and limitation of motion returned gradually. These observations could not be repeated.

Blood diseases: Two subjects with erythremia had elevated erythrocyte glycine; in one, receiving urethane, the plasma value was low. Elevated red cell glycine was also found in two patients with chronic myelogenous leukemia. In one (No. 92) the concentration decreased in both blood and plasma after the leucocytes had dropped from 230,000 to 12,900/mm. following treatment with urethane.[3]

One patient with untreated pernicious anemia showed normal glycine values.

Miscellaneous: High red cell glycine and amino N were observed in a case of pemphigus vulgaris (No. 81) (under nirvanol treatment); red cell glycine was also elevated in disseminated lupus and urticaria. Significantly low values were observed in two cases of ulcerative colitis with malnutrition (Nos. 106, 107). In one (No. 106) plasma glycine was low whereas the level in whole blood was normal. In the other subject (No. 107) blood and red cell glycine were low but the concentration in the plasma was normal. High red cell glycine levels were observed in a case of ruptured intervertebral disc (No. 94), and in one patient with carcinoma of the pancreas (No. 103).

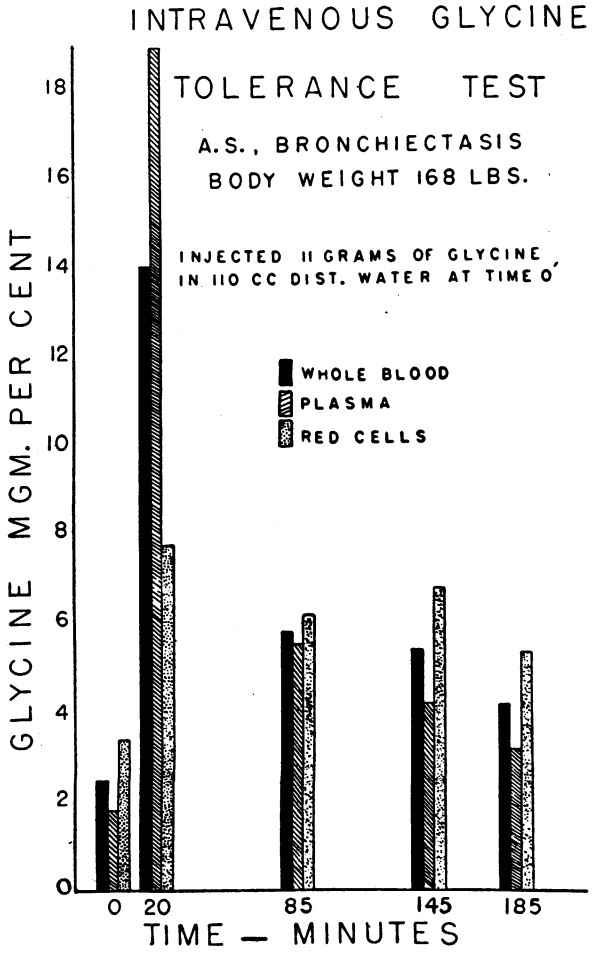

FIG. 1. EFFECTS OF INTRAVENOUSLY ADMINISTERED GLYCINE

Effects of intravenously administered glycine

No untoward reactions occurred following intravenous glycine. Within ten minutes the subjects generally experienced warmth and flushing of the face, and sometimes paresthesiae in fingers and toes. Because glycine is said to affect the kidneys of animals adversely (19), the urine was examined in all subjects during and after the test, but no changes were observed.

Following glycine infusion, blood values changed rather uniformly (Figure 1 is typical). Twenty minutes after injection plasma and red cell glycine became markedly elevated, the former to a greater extent than the latter. Subsequently plasma glycine declined gradually, sometimes falling to lower values than whole blood glycine since red cell glycine lagged behind plasma changes and, in some cases, remained elevated (Table II, No. 10). In only one subject (Table II, Nos. 10, 11) did plasma glycine return to the pre-injection level during the period of observation.

The height of the 20-minute rise in blood and plasma glycine seemed to be related to the fasting values; this relationship was not apparent for erythrocyte glycine.

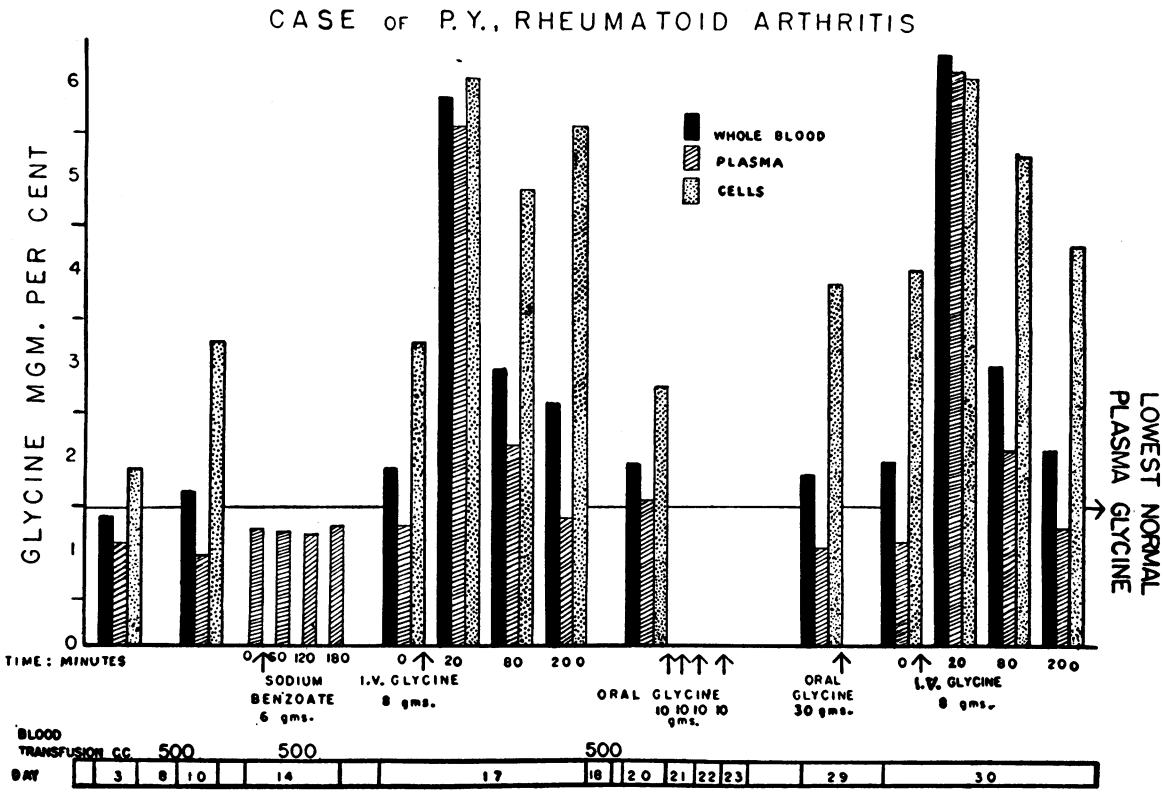

FIG. 2. INTRAVENOUS GLYCINE TOLERANCE TEST

Total glycine excreted during the test, always less than a gram, varied widely and was unrelated to the blood glycine curve.

The slow rise in erythrocyte glycine compared with plasma glycine may be due either to its slow diffusion into red cells or to its early transformation or conjugation within the cells. Experiments exploring these possibilities are presented below.

Oxalated blood, divided into four samples, was treated as follows:

Sample 1: 24 cc. were mixed with 0.5 cc. of an aqueous glycine solution (1.16 gms. per 100 cc.). The mixture was immediately analyzed for blood, "plasma," and red cell glycine.

Sample 2: 24 cc. were mixed with 0.5 cc. physiological saline; an aliquot was similarly analyzed.

Sample 3: to 12.8 cc. of plasma was added 0.5 cc. glycine solution; glycine was determined immediately.

Sample 4: to 12.8 cc. plasma was added 0.5 cc. isotonic sodium chloride solution and the mixture was analyzed.

Determinations were repeated two and four hours later on samples of the four mixtures which were incubated at 37° C. with frequent shaking. The results (Table III) show that in *Sample 2* the glycine of blood, plasma and erythrocytes rose slowly during incubation. The highest increase occurred in the cells where, it seemed, some glycine was either liberated from a conjugated form or was otherwise evolved. In *Sample 1* the artificially augmented plasma glycine declined slightly while cell glycine increased considerably. The total glycine of the mixture, however, rose only by an amount roughly equal to that observed in *Sample 2.*

TABLE III

Changes in plasma and red cell glycine in vitro

Hematocrit was 45 per cent packed cells and it remained constant. All values expressed in mgm. per cent. Incubated at 37° C.

Incubation time	*Sample 1* Blood +glycine			*Sample 2* Blood +saline			*Sample 3* Plasma +glycine	*Sample 4* Plasma +saline
	Blood	Plasma	Cells	Blood	Plasma	Cells		
hrs.								
0	25.2	41.9	4.69	2.17	1.75	2.68	41.7	1.91
2	25.6	40.2	7.71	3.08	1.99	4.41	41.9	1.72
4	27.2	38.6	13.20	3.57	2.34	5.09	43.4	1.94

DISCUSSION

The relatively normal plasma glycine concentrations in acute infectious hepatitis and cirrhosis of the liver are in accord with the normal total amino acid levels reported in these diseases (20–24). Only in complete hepatectomy (25) or severe liver damage (20) as in acute yellow atrophy (26–28) are amino acid values increased. The low glycine found in our case of subacute yellow atrophy might be referable to inability to synthesize this "non-essential" amino acid or to malnutrition, as suggested by the low plasma glycine observed in chronic ulcerative colitis with malnutrition. However, blood amino acid concentrations in experimental nutritional hypoproteinemia are normal (29) while our case of nutritional hypoproteinemia (Table I, Nos. 67–71) showed exceptionally high blood and plasma glycine levels after a low initial value.

The normal glycine tolerance in liver disease also is in agreement with the experience of others who found amino acid-loading tests useless in the diagnosis of liver disease (20, 23).

The high glycine concentrations in chronic glomerulonephritis is not related to the hypoproteinemia, the hyperaminoacidemia (30, 31) or the nitrogen retention found in kidney disease. Nor can the glycinemia be explained by inadequate urinary glycine excretion since the amount thus eliminated is usually small. It is possible that the metabolism of glycine in the diseased kidney is disturbed in view of the function of this organ in elaborating guanidoacetic acid from glycine and arginine (7, 32). The normal glycine tolerance in two nephritics, one with the nephrotic syndrome, is in accord with the reported undisturbed utilization of glycine in nephrosis (33).

The literature on blood amino acids in endocrine disturbances is extensive (34, 35). Thyroidectomy or thiouracil lowers blood amino acids in rats whereas tissue amino acids are unaffected. Okada and Hayashi (36), on the other hand, found no such alterations following thyroidectomy.

Our observations indicate that hypometabolism tends to be associated with high blood glycine and normal α amino N. The abnormally elevated plasma amino acid curve reported by Witts (21) after glycine administration in a patient with myxedema, is in accord with our results with the glycine tolerance test in hypometabolism. Presumably the tissues of these cases with high fasting plasma glycine are also rich in glycine and accordingly are less able to take up additional amounts of the amino acid.

Contrary to what might be expected from the above, glycine values were generally normal in hypermetabolism. This is in agreement with the blood amino N findings of Maddock *et al.* (37) but is in contrast to the elevated values reported for the plasma, liver, and skeletal muscles of rats treated with thyroxine (35). The low glycine and low oral glycine tolerance test (16) in one case of hyperthyroidism with severe myopathy and creatinuria may be related to this complication.

The elevated glycine in the acromegalic may be referable to the anabolic effect of the growth-promoting hormone of the pituitary on protein metabolism although this hormone is said to lower the blood amino acids (38–40).

The high glycine observed in a case of Addison's disease with hypotension and hypochloremia despite DOCA and salt therapy is noteworthy in view of decreased plasma amino acids in adrenalectomized rats, which increased markedly after administration of cortical extract (35).

The relation between glycine and creatine and the role of the latter in the metabolism of muscle have been studied extensively (7, 11, 12, 41–55). It is likely that in the myopathy of thyrotoxicosis (44) and in myopathies in general (34) there is no impairment of creatine synthesis, but rather of creatine storage and utilization. This concept is compatible with the low glycine values obtained in the patient with thyrotoxicosis and myopathy and in the subject with progressive muscular dystrophy if we assume that excessive creatinuria serves to deplete glycine stores faster than the amino acid can be synthesized in the body.

The elevated glycine and total blood amino acids in one patient with myasthenia gravis who was receiving methyltestosterone may be referable to the anabolic effects of this hormone (56).

Little which is non-speculative can be said concerning the strikingly low glycine in certain cases of rheumatoid arthritis. It is noteworthy that the glycine content of elastin, an important protein constituent of ligaments and tendons, is 25.5 per cent (57). The low tolerance curve and rapid clearance of injected glycine from the plasma of

one subject suggest an undue avidity of the tissues for this amino acid or its rapid destruction. The latter seems more likely since large amounts of glycine administered twelve hours prior to the test had no effect on the tolerance. In addition a hippuric acid test, to be reported elsewhere (58), revealed that hippuric acid excretion following ingestion of benzoate was subnormal in this patient. Further investigation of the role of glycine in this disease is in progress.

The high erythrocyte glycine in both polycythemia vera and myelogenous leukemia was striking. The decrease in glycine in myelogenous leukemia consequent to urethane therapy and the strikingly low plasma glycine in the polycythemic, who, under treatment with the same chemical, showed at the same time a significantly high erythrocyte glycine warrant further study of glycine metabolism and the effects of urethane in those blood dyscrasias.

Following the intravenous administration of 1 gm. of glycine per 15 lbs. of body weight plasma glycine rises to between 14 and 38 mgm. per cent. Assuming a plasma volume of 3.5 liters in patient A. S. the injection of 11 gms. of the amino acid should theoretically have resulted in a plasma glycine of about 300 mgm. per cent if all the glycine had remained in the plasma. After 20 minutes, only about 7 per cent of the injected amino acid could be accounted for in this patient's blood. These discrepancies in view of the relatively insignificant urinary glycine, indicate a rapid uptake of the amino acid by the tissues. Similar observations, based upon total amino N determination, have been made after administration of glycine or of amino acid mixtures (10–13, 59). In this respect it is significant that the amino acid levels in muscles and kidney remain high for more than three hours after glycine injection (60), whereas liver amino acid (60) and blood glycine had already returned almost to normal. Furthermore, in our experiments with benzoate-glycine conjugation (58), we observed that the ingestion of 10 gms. of glycine 12 hours prior to benzoate administration increases the amount of hippuric acid formed, despite the fact that plasma glycine is normal at the time of the test. The supplementary glycine probably was present in the tissues, either free or bound in an easily available form.

After the ingestion of protein plasma and erythrocyte amino acids rise, the latter lagging behind the former and persisting for several hours even when plasma amino acid was returning toward normal (61). Similar observations regarding glycine have been described after oral administration of this amino acid (16, 62). The same results are obtained following intravenous glycine. On the basis of our *in vitro* experiments this lag is best interpreted as being due to a slow diffusion of glycine into and out of the erythrocytes.

SUMMARY

Blood glycine and α amino N have been studied in various pathological states. Low blood glycine was observed in one case of subacute yellow atrophy, one subject with hyperthyroidism with myopathy, and in two cases of ulcerative colitis. The lowest values were observed in subjects with rheumatoid arthritis.

A distinct tendency toward elevated glycine, particularly in the erythrocytes, was found in some patients with liver disease, with chronic glomerulonephritis, with hypometabolism, with polycythemia vera, and with leukemia.

No relation was evident between the concentrations of glycine, total α nitrogen, plasma protein, and blood non-protein nitrogen.

The intravenous administration of glycine results in a rapid rise and subsequent decline in both plasma and red cell glycine concentration. The latter lags somewhat behind the former, which, on the basis of *in vitro* studies, is interpreted as being due to slow diffusion of glycine into and out of the erythrocyte.

Certain abnormalities in the glycine tolerance curve were observed in a few pathological subjects.

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