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Research Article

THE GLYCINE TOLERANCE TEST IN SPRUE AND PERNICIOUS ANEMIA

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Fairley (1) proved by the results of glucose tolerance tests, and Barker and Rhoads (2) by fat tolerance tests, that the absorption of sugar and fat by patients with sprue is improved after treatment with liver extract. Moreover, Groen (3) observed that the diminished rate of absorption of glucose by the intestinal tract of 3 patients with pernicious anemia became normal after similar therapy.

From these observations it was concluded that in both sprue and pernicious anemia a dysfunction of the intestinal tract is present which may be corrected by some constituent of liver extract. These conclusions were not entirely warranted, however, since no proof was advanced that simple diarrhea, or ischemia of the intestine, unrelated to liver extract deficiency, would not result in a similar interference with absorption. Moreover, the evidence concerning the malabsorption of glucose and of fat is repeatedly advanced as proof that the effective principle of liver extract is also poorly absorbed. No adequate experimental data bearing on this point are at hand, however, since the studies of Dakin, Ungley, and West (4) and of Subbarow, Jacobson, and Hartfall (5) indicate that the active substance is a breakdown product of protein, probably a peptide. Accordingly, studies have been made of the absorption of an amino acid, glycine, by patients with sprue and pernicious anemia, as well as with other disorders. The results are presented in this communication.

The glycine tolerance test was employed to obtain information concerning both the rate of absorption of the amino acid and the speed with which it is metabolized. Studies were made before and after the intramuscular administration of liver extract to 2 patients with sprue and to 5 patients with pernicious anemia. Twelve other patients—3 with pernicious anemia in induced remissions, 4 with ulcerative colitis, 3 with refractory anemia, and 2 with hepatic cirrhosis—were subjected to single tests and served as control cases.

The tests were made by the measurement of the levels of the plasma amino nitrogen and the plasma and urinary urea nitrogen before and after the ingestion of glycine. The levels of the plasma amino nitrogen are considered to indicate the rates of absorption and deaminization of the amino acid, whereas the levels of the plasma and urinary urea nitrogen indicate the rates of urea formation and excretion. Kirk (6) has reviewed the evidence that most, if not all, of the urea in the blood is formed in the liver, and that absorbed amino acids are deaminized by that organ as well as by the kidneys. He also showed by the results of glycine tolerance tests that patients with acute uremia are not able to deaminize glycine as rapidly as normal individuals or as patients with chronic uremia.

Heath and Fullerton (7) studied the levels of the plasma amino nitrogen after the oral as well as the intravenous administration of glycine. They concluded that "the amino acid nitrogen content of the blood following the ingestion of 25 grams of glycine gives no useful information regarding the rate of absorption from the gastro-intestinal tract." It should be noted that the studies upon which these conclusions were based did not include determinations of the plasma urea nitrogen levels. Hence no information concerning the rate of deaminization was available. If this were low, a plasma amino nitrogen curve within normal limits might follow the administration of glycine, even though absorption was impaired. Furthermore, no information is presented regarding the blood levels of the 2 tested patients with pernicious anemia in relapse.

METHODS

The glycine tolerance test (8) was made by the oral administration of 25 grams of glycine dissolved in 500 cc. of water to the fasting patient

at 9 A.M. Blood samples were withdrawn before the amino acid ingestion, and at 10 A.M., 11 A.M., 12 noon, 1 P.M., and 3 P.M. Urine was collected from 9 A.M. to 12 noon, and from 12 noon to 3 P.M.

Plasma amino nitrogen and plasma and urinary urea nitrogen levels were determined by the methods of Van Slyke (9). The normal plasma amino nitrogen and plasma urea nitrogen curves after the ingestion of glycine, as established by Kirk and Witts (6, 8), are represented in each figure (Figures 1 to 7). Kirk also has shown that normal fasting individuals after the ingestion of 25 grams of glycine (or 4670 mgm. of amino nitrogen) excrete in the urine approximately 2000 mgm. of urea nitrogen during the first 3 hours, and approximately 1000 mgm. during the second 3 hours. The total amount of urea formed during the test is ascertained from the total output of urea in the urine plus the increase of the urea content of the body.

Liver function tests

a. Urobilinogen excretion in the urine. The Watson-Terwen (10) method was used. The excretion of urobilinogen in the urine of 25 normal individuals was found to be less than 2 mgm. a day.

b. The bilirubin excretion test described by Harrop and Barron (11) was made by injecting intravenously 1 mgm. of crystalline bilirubin per kilo of body weight. A retention after 4 hours of more than 5 per cent of the injected pigment was regarded as evidence of hepatic dysfunction.

c. The sodium benzoate conversion test described by Quick (12) was made by administering orally 5.9 grams of sodium benzoate. An excretion in the urine in 4 hours of less than 3 grams of hippuric acid in the presence of normal renal function was regarded as evidence of hepatic dysfunction.

Renal function tests

The urea clearance test was used (13). The expected clearance is between 70 and 120 per cent of a normal standard.

Examination of the blood

Oxalated venous blood and standardized pipettes and chambers were used. Hemoglobin was determined by the Sahli method with a glass standard and calibrated tubes.

CASE HISTORIES

1. Patients with sprue

Case 1. N. D., female, aged 59. Hospital Number 10591. Admitted November 7, 1938, complaining of weakness, loss of weight, diarrhea, and pallor (see Figure 1). The patient had been a missionary for 12 years in China and the Philippine Islands. She contracted diarrhea and returned to this country with a diagnosis of sprue 3 years before admission. The symptoms were controlled by the administration of liver extract but 6 months previous to admission the therapy was discontinued, and she was admitted in a severe relapse. She was a pale, sallow woman who appeared to be chronically ill. The tongue was reddened and showed marked papillary atrophy. The abdomen was distended with gas. Free hydrochloric acid was present in the fasting gastric juice after injection of histamine. The red blood cell count was 850,000 and the hemoglobin 28 per cent. Urea clearance test was 85 per cent of normal. Bilirubin excretion test showed 20 per cent retention; sodium benzoate conversion test, 1.3 grams; daily urinary urobilinogen output, 2.5 to 5.0 mgm. The patient was given approximately 20 cc. of liver extract intramuscularly during the interval between the glycine tolerance tests. A typical reticulocyte response (17.5 per cent) was followed by clinical recovery. At the time of the second glycine test the red blood cell count was 3,530,000, hemoglobin 71 per cent. Urea clearance was 80 per cent. Bilirubin excretion test, 5 per cent retention; sodium benzoate conversion test, 2.8 grams, daily urinary urobilinogen output, 0.8 mgm.

Case 2. A. V., male, aged 58. Hospital Number 10386. Admitted February 22, 1938, complaining of weakness, pallor, and 3 or 4 foamy ill-smelling stools daily (see Figure 1). The patient was born in Spain, lived in Cuba for 14 years and in the United States for the last 20 years. He first developed diarrhea in 1934 and the symptoms were controlled with blood transfusions and liver extract. No therapy was given for 18 months before admission and the attack during which he was admitted was the fourth and most severe. He was a small, poorly nourished, emaciated male, obviously exhausted and mentally depressed. Tongue papillae were slightly atrophic, and the abdomen was markedly distended with gas. Pitting edema of the ankles was present. The red blood cell count was 1,350,000; hemoglobin 36 per cent. Urea clearance was 70 per cent of normal. Bilirubin excretion test showed 68 per cent retention; sodium benzoate conversion test, 1.2 grams; daily urinary urobilinogen, 4.9 mgm. No free hydrochloric acid was present in the fasting gastric juice even after the injection of histamine. A gastro-intestinal series showed the small bowel to be spastic and tubular, the large bowel atonic and distended. In the interval between the 2 glycine tolerance tests 70 cc. of liver extract were given

intramuscularly. The number of reticulocytes rose to 10.8 per cent, and clinical recovery followed. At the time of the second glycine test the red blood cell count was 3,580,000, hemoglobin 87 per cent. Urea clearance was 85 per cent of normal. Bilirubin excretion test, 2 per cent retention; sodium benzoate conversion test, 3.1 grams; daily urinary urobilinogen, 1.0 mgm.

2. Patients with pernicious anemia (not previously treated)

Case 3. M. C., female, aged 40. Hospital Number 10616. Admitted December 5, 1938, complaining of weakness, pallor, palpitation and sore gums gradually increasing for 1½ years (see Figure 2). She was a fair, well developed, pale individual. The papillae of the tongue were atrophic and the vibratory sense was intact. The red blood cell count was 980,000, hemoglobin 31 per cent. Urea clearance was 180 per cent of normal. Daily urinary urobilinogen was —15.0 to 65.0 mgm.

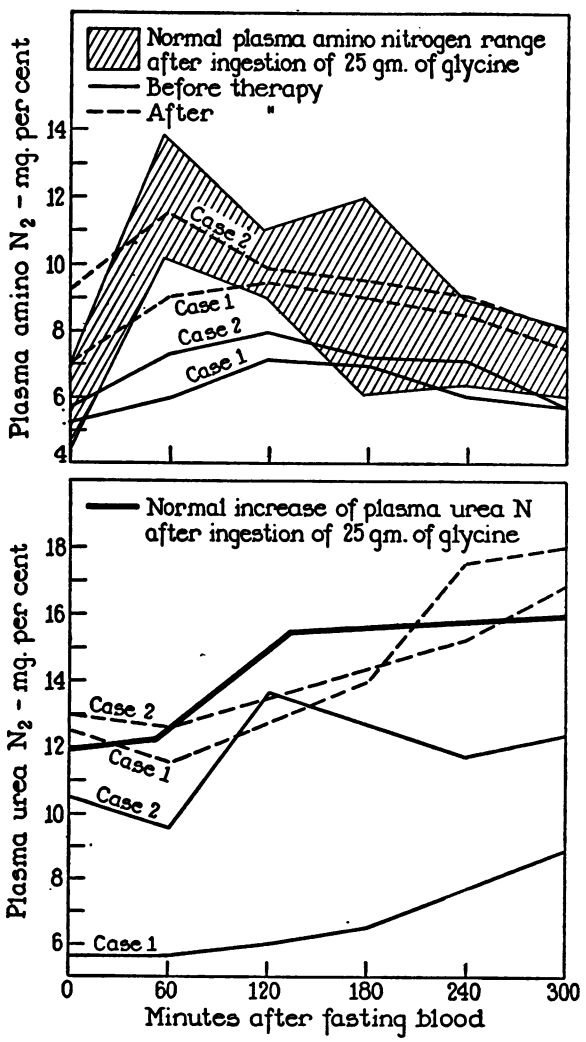

FIG. 1. SPRUE

There was no free hydrochloric acid in the gastric juice even after the injection of histamine. The sternal bone marrow was hyperplastic and the number of megaloblasts was markedly increased. After the first glycine tolerance test was made, the patient was given 20 cc. of liver extract intramuscularly and the reticulocytes rose to 30 per cent, followed by a rapid increase in the number of erythrocytes. The second test was made 5 months after treatment when clinical recovery was complete. The red blood cell count was 3,700,000, hemoglobin 76 per cent. Urea clearance was 100 per cent of normal. Daily urinary urobilinogen was 3.5 mgm.

Case 4. M. H., male, aged 56. Hospital Number 10512. Admitted July 21, 1938, complaining of weakness, pallor, anorexia, and numbness and tingling of the lower extremities (see Figure 2). The history was irrelevant until 6 months previous to admission when he noticed gradually increasing weakness associated with cramps in the calves of the legs and in the feet. Two months later numbness appeared in the feet, and gradually ascended to his knees. Anorexia appeared later. He was a pale, well developed male, with slight atrophy of the papillae of the tongue. The oral mucous membranes were pale and the vibratory sense was diminished. The Romberg test was slightly positive. The red blood cell count was 1,640,000, hemoglobin 44 per cent. Urea clearance was 28 per cent. Bilirubin excretion test, 3.9 per cent retention; sodium benzoate excretion, 2.5 grams. The gastric juice contained no free hydrochloric acid even after the injection of histamine. The sternal bone marrow was hyperplastic with a predominance of mega-

FIG. 1. SPRUE

Before therapy

	Red blood cells	Hemoglobin	Color index	Total urea formation	Urea clearance test	Liver function tests (urinary urobilinogen test; Harrop, Barron and Quick tests)
		per cent		*mgm.*	*per cent of normal*	
Case 1	850,000	28	1.7	2180	85	2.5 and 5.0 mgm. urinary urobilinogen daily 20.0 per cent retention of bilirubin 1.3 grams of hippuric acid excreted in 4 hours
Case 2	1,350,000	38	1.38	2870	70	4.9 mgm. urinary urobilinogen daily 68.0 per cent retention of bilirubin 1.2 grams of hippuric acid excreted in 4 hours
				After therapy		
Case 1	3,530,000	71	1.0	3492	80	0.8 mgm. urinary urobilinogen daily 5.0 per cent retention of bilirubin 2.8 grams of hippuric acid excreted in 4 hours
Case 2	3,580,000	87	1.2	3161	85	1.0 mgm. urinary urobilinogen daily 2.0 per cent retention of bilirubin 3.1 grams of hippuric acid excreted in 4 hours

loblasts. X-ray of the gastro-intestinal tract was normal. The Wassermann reaction was negative. Ten days following the first glycine test the patient experienced a transitory renal disorder. It was characterized by chills, temperature of 100°, many hyaline casts in the urine, and tenderness in the left kidney region. The urea clearance was 20 per cent of normal at that time. All evidence of this disorder disappeared after 48 hours, and no more attacks occurred during the 13 months that the patient was followed. The reticulocyte count rose to 12.4 per cent on the tenth day following the first injection of liver extract, and 70 cc. of liver extract were given during the 3 months between tests. Clinical recovery was complete. At the time of the second test the red blood cell count was 4,980,000, hemoglobin 107 per cent. Urea clearance was 105 per cent of normal.

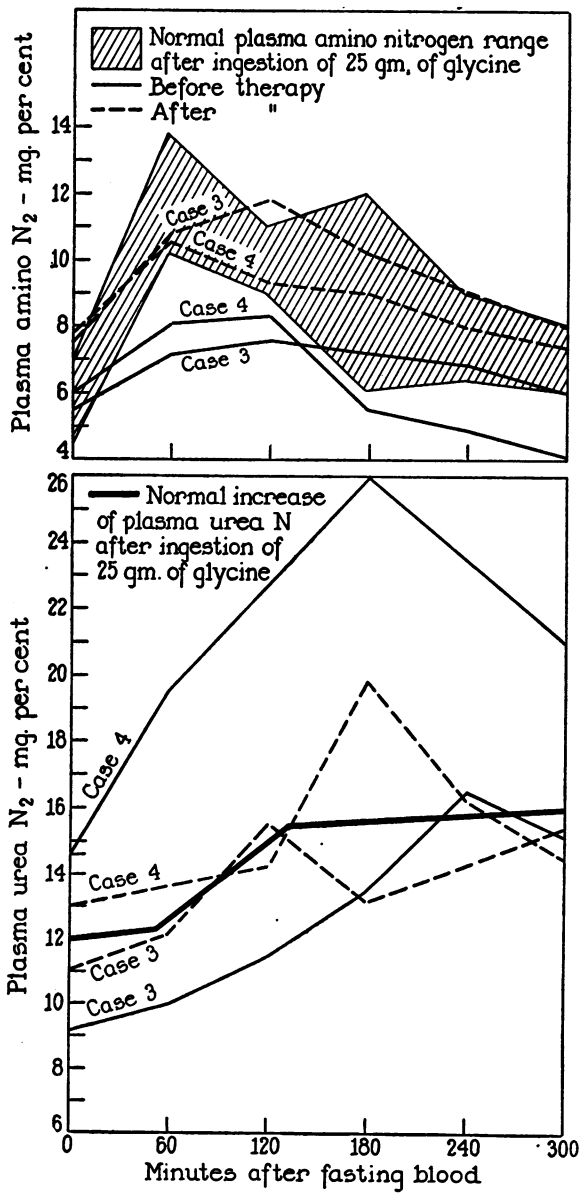

FIG. 2. PERNICIOUS ANEMIA, NOT PREVIOUSLY TREATED

3. Patients with pernicious anemia (previously treated)

Case 5. E. McK., female, aged 50. Hospital Number 10221. Admitted on July 28, 1937, complaining of weakness, pallor, numbness and tingling of the arms and legs, and ataxia (see Figure 3). One year previous to admission the patient had noted anorexia and difficulty in walking. She was treated with liver extract and a remission occurred, but therapy was discontinued and she was admitted in a relapse. She was a well developed, well nourished, rather pale woman. The lingual papillae were atrophic at the tip, the vibratory sense was absent, and sensation to light touch was diminished. The red blood cell count was 2,060,000, hemoglobin 43 per cent. Urea clearance was 100 per cent of normal. No free hydrochloric acid was present in the gastric juice even after the injection of histamine. The gastro-intestinal series was negative. After the first glycine test liver extract was administered and a reticulocytosis of 12 per cent occurred. The clinical remission was gradual but complete. At the time of the second test the red blood cell count was 4,320,000, hemoglobin 90 per cent. Urea clearance was 100 per cent of normal.

Case 6. M. K., female, aged 67. Hospital Number 10456. Admitted April 29, 1938, complaining of weakness, blurred vision, numbness, tingling of finger tips, and pallor (see Figure 3). Three years before the patient had noted gradually increasing weakness and pallor. Later blurred vision and numbness in the finger tips developed and liver extract was administered. Improvement followed, but therapy was discontinued and a relapse ensued. Lextron had been taken before admission without clinical effect. She was well developed but slender. In the retinal vessels numerous sclerotic areas were visible. The oral mucous membranes were normal and the vibratory sense was poor. The red blood cell

FIG. 2. PERNICIOUS ANEMIA—NOT PREVIOUSLY TREATED

Before therapy

	Red blood cells	Hemoglobin	Color index	Total urea formation	Urea clearance test	Liver function tests (urinary urobilinogen test; Harrop, Barron and Quick tests)
		per cent		*mgm.*	*per cent of normal*	
Case 3	980,000	31	1.6	2714	180	65.0–15.0 mgm. urinary urobilinogen daily
Case 4	1,640,000	44	1.3	1750	28	3.9 per cent retention of bilirubin 2.5 grams of hippuric acid excreted in 4 hours
				After therapy		
Case 3	3,700,000	76	1.0	3921	100	3.5 mgm. urinary urobilinogen daily
Case 4	4,980,000	107	1.0	2835	105	

count was 2,880,000, hemoglobin 63 per cent. Urea clearance was 60 per cent of normal. Bilirubin excretion test, 2.0 per cent retention; sodium benzoate excretion, 2.9 grams; daily urinary urobilinogen, 0.8 mgm. Four degrees of free hydrochloric acid were present after injection of histamine. The sternal bone marrow was hyperplastic and a differential count revealed an increased number of normoblasts and erythroblasts. After the administration of liver extract the reticulocyte count rose to 8.8 per cent. The clinical remission which followed was gradual but complete. At the time of the second glycine test the red blood cell count was 4,170,000, hemoglobin 87 per cent. Urea clearance was 90 per cent of normal.

Case 7. J. B., male, aged 40. Hospital Number 9757.

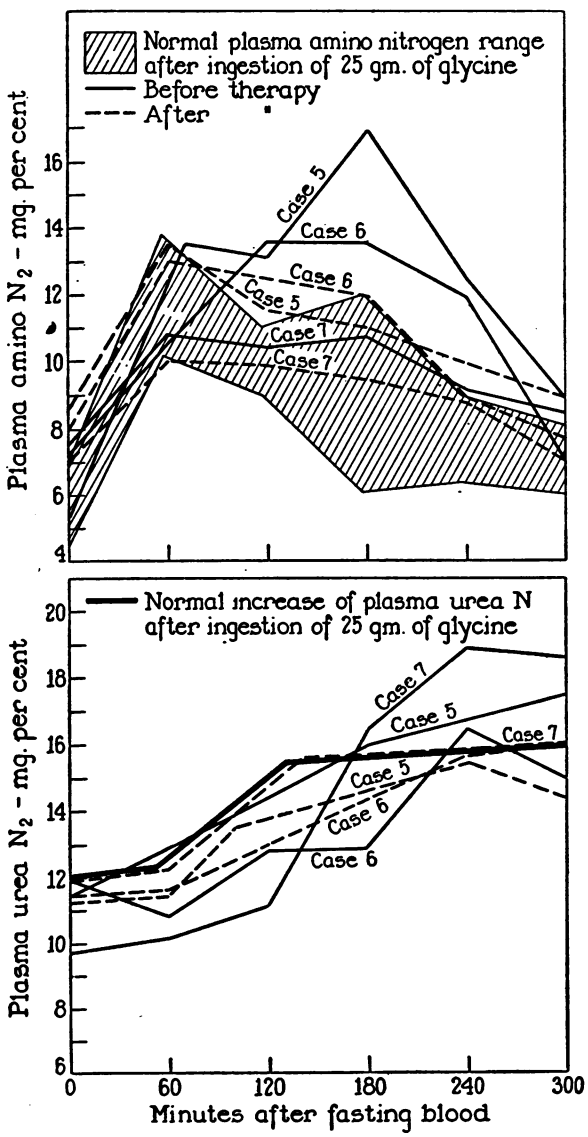

FIG. 3. PERNICIOUS ANEMIA, PREVIOUSLY TREATED

Admitted on February 11, 1938, complaining of weakness, pallor, shortness of breath, glossitis, and occasional paresthesias of the lower extremities (see Figure 3). The patient had had 2 induced remissions of his disorder before his admission. He was well developed, and well nourished, with a yellowish pallor of the skin. The tongue was diffusely reddened but the papillae were not atrophic. The vibratory sense was diminished. The red blood cell count was 2,470,000, hemoglobin 69 per cent. Urea clearance was 120 per cent of normal. Bilirubin excretion test, no retention; sodium benzoate test, 3.2 grams; daily urinary urobilinogen, 2.5 mgm. No free hydrochloric acid was present in the fasting gastric juice. The sternal bone marrow was hyperplastic, with an abnormally increased number of megaloblasts and erythroblasts. The gastro-intestinal series was negative. After the administration of liver extract there was a reticulocytosis of 16 per cent, and complete recovery followed. When the second glycine test was made the red blood cell count was 3,540,000, hemoglobin 84 per cent. Urea clearance was 100 per cent of normal.

4. Patients with pernicious anemia in induced remissions

Case 8. T. T., male, aged 60. Hospital Number 10355. Admitted February 1, 1938, complaining of severe weakness and pallor. The patient had had 3 induced remissions before admission. He received 5 cc. of liver extract weekly for 6 months before the glycine test was made. At the time of the test the red blood cell count was 4,340,000, hemoglobin 90 per cent, and white blood cell count 6,900. The differential count was normal. Bilirubin excretion test, 6 per cent retention; sodium benzoate test, 3.93 grams. Urea clearance was 100 per cent of normal.

Case 9. C. M., female, aged 71. Hospital Number 10085. Admitted March 6, 1937, complaining of weakness

FIG. 3. PERNICIOUS ANEMIA—PREVIOUSLY TREATED

Before therapy

	Red blood cells	Hemoglobin	Color index	Total urea formation	Urea clearance test	Liver function tests (urinary urobilinogen test; Harrop, Barron and Quick tests)
		per cent		*mgm.*	*per cent of normal*	
Case 5	2,060,000	43	1.0		100	
Case 6	2,880,000	63	1.1	1911	60	0.8 mgm. urinary urobilinogen daily 2.0 per cent retention of bilirubin 2.9 grams of hippuric acid excreted in 4 hours
Case 7	2,470,000	69	1.4	2774	120	2.5 mgm. urinary urobilinogen daily 0.0 per cent retention of bilirubin 3.2 grams of hippuric acid excreted in 4 hours

After therapy

Case 5	4,320,000	90	1.0	2827	100	
Case 6	4,170,000	87	1.0	2974	90	
Case 7	3,540,000	84	1.4	3676	100	

and pallor. Red blood cell count 1,400,000, hemoglobin 50 per cent, and white blood cell count 5,540. The differential count was normal. Ten days following the administration of liver extract the reticulocyte count rose from 0.5 per cent to 10.0 per cent. Following weekly intramuscular administrations of 5 cc. of liver extract for 5 months the glycine test was made. At that time the blood findings were: red blood cell count 3,310,000, hemoglobin 70 per cent, and white blood cell count 4,600. The daily urinary urobilinogen output was 0.5 mgm.

Case 10. B. M., female, aged 73 years. Hospital Number 10243. Admitted October 12, 1937, complaining of severe weakness and pallor. Red blood cell count 1,100,000, hemoglobin 27 per cent, white blood cell count 6,500. Differential count was normal. The patient was desperately ill and liver extract was administered on admission. The reticulocytes rose to 16 per cent in 10 days. When the glycine test was made following the weekly intramuscular administration of 5 cc. of liver extract for 5 months, the red blood cell count was 3,600,000, hemoglobin 78 per cent, and the white blood cell count 5,400. Bilirubin excretion test, 5.0 per cent retention; sodium benzoate conversion test, 1.8 grams; daily urinary urobilinogen, 0.8 mgm. Urea clearance was 40 per cent of normal. As estimated from the results of the urea clearance test, the renal function was below normal levels and the consequent retention of hippuric acid may have caused the apparently poor conversion of sodium benzoate.

5. *Patients with refractory anemia*

Case 11. V. G., female, aged 48. Hospital Number 9871. Admitted on 6 different occasions between August 1937 and April 1939. The patient had had typical aplastic anemia since 1935 requiring transfusions about every 3 months. The glycine tolerance test was made when the red blood cell count was 1,240,000, hemoglobin 27 per cent, white blood cell count 3,100, and platelets 80,000. The bone marrow obtained by biopsy and later by puncture was hypoplastic. Except for a slight lymphocytosis the differential count was normal. Bilirubin excretion test, 5 per cent retention; sodium benzoate conversion test, 3.4 grams. Urea clearance was 85 per cent of normal. Daily urobilinogen output in feces and urine was abnormally low (45 mgm. and 0.1 mgm. respectively). The patient was very comfortable and active as long as she received 500 cc. of blood every 2 or 3 months.

Case 12. B. R., male, aged 62. Hospital Number 10602. Admitted November 21, 1938, complaining of weakness and pallor of 12 to 18 months' duration. The patient was without lymphadenopathy or splenomegaly. The red blood cell count was 1,030,000, hemoglobin 24 per cent, white blood cell count 2,100, platelets 100,000. The differential count was: polymorphonuclears 52 per cent, lymphocytes 43 per cent, monocytes 5 per cent. The bone marrow was hyperplastic and contained young myeloid and erythroid cells. The fragility test was normal. Bilirubin excretion test, 11.5 per cent retention; sodium benzoate conversion test, 3.9 grams. Urea clearance was 60 per cent of normal. The daily output of urobilinogen in feces (73 mgm.) and urine (0.3 mgm.) was below normal. The patient was given 6 transfusions of 500 cc. of blood each during a period of 5 months. Numerous types of therapy, including liver extract, failed to raise the blood levels. He died 6 months after admission.

Case 13. H. F., female, aged 54. Hospital Number 10394. Admitted February 28, 1938, complaining of weakness, pallor and shortness of breath. Red blood cell count was 1,480,000, hemoglobin 42 per cent, white blood count 3,250, platelets 80,000. Differential count: polymorphonuclears 34 per cent, eosinophils 2 per cent, lymphocytes 46 per cent, monocytes 18 per cent. Fragility test was normal. Bilirubin excretion test, 8.3 per cent retention; sodium benzoate conversion test, 2.56 grams. Urea clearance test was 75 per cent of normal. The output of urobilinogen in feces (80 mgm.) and urine (0.4 mgm.) was less than normal. Bence-Jones proteinuria was not present. Innumerable small areas of rarefaction were revealed by x-ray examination of the long bones and skull. She died of multiple myeloma 14 months after admission.

6. *Patients with ulcerative colitis*

Case 14. A. S., female, aged 52. Hospital Number 10466. Admitted April 29, 1938, complaining of severe diarrhea of 30 years' duration. The diagnosis of ulcerative colitis had been made in many clinics. The red blood cell count was 4,380,000, hemoglobin 86 per cent, white blood cell count 8,600. Differential count: polymorphonuclears 64 per cent, eosinophils 2 per cent, lymphocytes 18 per cent, monocytes 16 per cent. Blood, pus, yeast cells, and bacteria, but no amoebae, were found in the feces. X-ray examination of the gastro-intestinal tract after a barium enema revealed a smooth-walled, nonplicated, spastic colon and lower ileum. During hospitalization the patient had between 8 and 14 watery foul-smelling bowel movements a day.

Case 15. R. J., male, aged 35. Hospital Number 10044. Admitted February 4, 1937, complaining of weakness and diarrhea of 10 years' duration. The stools contained pus and blood. Red blood cell count was 3,900,000, hemoglobin 84 per cent, white blood cell count 4,400. Differential count: polymorphonuclears 76 per cent, eosinophils 3 per cent, basophils 2 per cent, lymphocytes 13 per cent, monocytes 6 per cent. Bilirubin excretion test, no retention; sodium benzoate test, 2.5 grams; galactose tolerance test, no excretion in 4 hours. The colon when observed by fluoroscopy was redundant and relaxed. During hospitalization the patient had from 2 to 7 watery, foul-smelling bowel movements daily. The patient died and autopsy revealed ulceration of the colon.

Case 16. L. C., male, aged 43. Hospital Number 10250. Admitted October 19, 1938, complaining of diarrhea of 2 years' duration. Blood and pus were periodically seen in the stools. The red blood cell count was 3,320,000, hemoglobin 32 per cent, white blood cell count

3,650. Differential: polymorphonuclears 84 per cent, eosinophils 3 per cent, basophils 1 per cent, lymphocytes 5 per cent, monocytes 7 per cent. Bilirubin excretion test, 12 per cent retention; sodium benzoate test, 1.5 grams; the urinary urobilinogen output rose to 214 mgm. a day. Urea clearance was 55 per cent of normal. By x-ray of the gastro-intestinal tract a mass partly obstructing the sigmoid was demonstrated as well as a fistula between the sigmoid and small bowel. During hospitalization the patient had between 2 and 6 stools daily which contained pus and blood. He was operated upon elsewhere and a carcinoma of the lower colon with diffuse ulceration of the sigmoid was found.

Case 17. M. S., female, aged 34. Hospital Number 10561. Admitted September 26, 1938, complaining of chronic diarrhea of 2 years' duration, weakness, and loss of weight. Red blood cell count was 5,370,000, hemoglobin 104 per cent, white blood cell count 8,550. The differential count was normal. Bilirubin excretion test, no retention; sodium benzoate test, 1.22 grams. Urea clearance was 20 per cent of normal. The reduced renal function may have been responsible for the abnormal results of the sodium benzoate test. During 3 months' hospitalization, the patient had from 4 to 10 watery foul-smelling stools daily containing pus and blood.

7. Patients with hepatic cirrhosis

Case 18. V. D., male, aged 57. Hospital Number 10406. Admitted March 8, 1938, complaining of weakness and enlarged abdomen. During 4 months of hospitalization 4 paracenteses were made. Approximately 10 liters of yellow fluid were withdrawn on each occasion. The red blood cell count was 4,760,000, hemoglobin 75 per cent, white blood cell count 3,750. Differential count: polymorphonuclears 72 per cent, eosinophils 2 per cent, lymphocytes 18 per cent, monocytes 8 per cent. Bilirubin excretion test, 50 per cent retention; sodium benzoate conversion test, 1.33 grams. The output of urobilinogen in the urine was consistently elevated and varied between 20 and 90 mgm. daily. At autopsy the liver was markedly cirrhotic.

Case 19. M. S., female, aged 53. Hospital Number 10361. Admitted first on February 4, 1938, complaining of weakness, shortness of breath, and an enlarged abdomen. During the 18 months that this patient was followed, 9 paracenteses were made. From 8 to 20 liters of yellow fluid were removed on each occasion. Two severe hematemeses occurred during that interval. The red blood cell count was 4,880,000, hemoglobin 83 per cent, white blood cell count 13,550. Differential count: polymorphonuclears 80 per cent, eosinophils 6 per cent, basophils 4 per cent, lymphocytes 6 per cent, and monocytes 4 per cent. Bilirubin excretion test, no retention; sodium benzoate conversion test, 2.2 grams. The output of urobilinogen in urine varied from 2.0 to 7.0 mgm. daily. Urea clearance was 80 per cent of normal. At autopsy, the liver was markedly cirrhotic.

RESULTS

The 19 patients were divided into 7 groups:

1. Two patients with sprue in relapse and previously treated (Cases 1 and 2).
2. Two patients with untreated pernicious anemia (Cases 3 and 4).
3. Three patients with pernicious anemia in relapse and previously treated (Cases 5, 6, and 7).
4. Three patients with pernicious anemia in induced remissions (Cases 8, 9, and 10).
5. Three patients with refractory anemia (Cases 11, 12, and 13).
6. Four patients with ulcerative colitis (Cases 14, 15, 16, and 17).
7. Two patients with hepatic cirrhosis (Cases 18 and 19).

1. Patients with Sprue (see Figure 1)

The glycine tolerance test was made on both patients (Cases 1 and 2) when the hemoglobin levels (28 per cent and 38 per cent) and the red blood cell levels (850,000 and 1,350,000) were low. The test was repeated after 5 and 2 months, respectively, of adequate liver extract therapy when the hemoglobin levels (71 per cent and 87 per cent) and the red blood cell levels (3,530,000 and 3,580,000) were nearer the normal. The patients, before treatment, were having 2 or 3 watery, offensive stools a day, but after treatment their bowel function was normal.

Before therapy. In both patients the plasma amino nitrogen curves were flat and low. The plasma urea nitrogen curves were also somewhat below those of normal individuals and during the 6 hours following the ingestion of glycine 2180 mgm. and 2870 mgm. respectively, of urea were formed. The results of the renal function tests were normal (70 per cent and 85 per cent), but those of the liver function tests were not. The first patient (Case 1) excreted daily in the urine an average of 2.3 to 5.0 mgm. of urobilinogen during several 3-day test periods, retained 20 per cent of the injected bilirubin in the plasma after 4 hours, and excreted only 1.3 grams of hippuric acid in the urine in a 4-hour period following the ingestion of 5.9 grams of sodium benzoate. The second patient (Case 2) excreted an average of

4.9 mgm. of urobilinogen, retained 68 per cent of the bilirubin and excreted 1.2 grams of hippuric acid.

After therapy. The plasma amino nitrogen curves were somewhat flattened but were within the normal range. The plasma urea nitrogen curves were essentially normal and 3492 mgm. and 3161 mgm. of urea were formed. The results of the renal function tests remained normal and the results of the liver function tests returned to normal. The first patient (Case 1) excreted 0.8 mgm. of urobilinogen, retained 5 per cent of bilirubin and excreted 2.8 grams of hippuric acid in the urine. The second (Case 2) excreted 1.0 mgm., retained only 2 per cent of the bilirubin and excreted 3.1 grams of hippuric acid in the urine.

2. *Two patients with untreated pernicious anemia (see Figure 2)*

The first glycine tolerance tests were made on both patients (Cases 3 and 4) when the hemoglobin levels (31 per cent and 44 per cent) and red blood cell levels (980,000 and 1,640,000) were low. The second tests were made 6 months later when the hemoglobin levels (76 per cent and 107 per cent) and red blood cell counts (3,700,000 and 4,980,000) were nearer normal.

Before therapy. Both plasma amino nitrogen curves were flattened and below normal. The plasma urea nitrogen curve of the first patient was also low (Case 3), and was associated with a total excretion of 2714 mgm. of urea and an elevated urea clearance (180 per cent). The plasma urea nitrogen values were high in the second patient (Case 4), due presumably to retention. Only 1750 mgm. of urea were formed and the renal function was but 28 per cent of normal. The results of the liver function tests were not strikingly abnormal: 3.9 per cent retention of bilirubin and 2.5 grams of hippuric acid excreted. The slightly decreased excretion of hippuric acid probably could be attributed to the reduced renal function, since 10 days later the patient suffered from a mild transitory renal disorder of which an early phase was undoubtedly present when the test was made (see case history).

After therapy. The results of the glycine tolerance tests were essentially normal in both patients. Case 3 formed 3921 mgm. of urea and the urea clearance was 100 per cent. Case 4 excreted 2835 mgm. of urea and the urea clearance was 105 per cent, a marked improvement over that present during the first test.

3. *Three patients with pernicious anemia previously treated (see Figure 3)*

This group was composed of patients in relapse (Cases 5, 6, and 7) who had been inadequately treated with liver extract before their hospitalization. The first glycine tolerance tests were made when the hemoglobin values (43 per cent, 63 per cent, and 69 per cent) and red blood cell levels (2,060,000, 2,880,000, and 2,470,000) were low. The tests were repeated after adequate liver extract therapy when the hemoglobin percentages were 90, 87, and 84, and the red blood cell counts were 4,320,000, 4,170,000 and 3,540,000, respectively.

Before therapy. The plasma amino nitrogen curves were normal in 1 patient (Case 7) and elevated in 2 (Cases 5 and 6). In 2 (Cases 6 and 7) the plasma urea nitrogen curves were somewhat low and the peaks were delayed, indicating a retarded urea formation. The total quantity of urea formed was 1911 mgm. in the patient (Case 6) with the greater retardation, and was 2774 mgm. in the other (Case 7). The third patient (Case 5) had a normal plasma urea nitrogen curve. The output of urea was not measured and the results of the renal and liver function tests were within the normal range.

CONTROL GROUPS

4. *Patients with pernicious anemia in induced remissions (see Figure 4)*

This group was composed of 3 patients with pernicious anemia (Cases 8, 9, and 10) who had received intramuscularly 5 cc. of liver extract[1] weekly for from 5 to 6 months previous to the glycine tolerance tests. The blood levels were essentially normal at the time of the tests (see case histories). The plasma amino nitrogen curve was normal in Case 9, essentially normal in Case 10, and slightly low in Case 8. The plasma urea nitrogen curve was normal in Case 8, but elevated in Cases 9 and 10. Unfortunately, the urea clearance test was not made on Case 9. In Case 10 it

[1] Eli Lilly and Company (unconcentrated liver extract for intramuscular use).

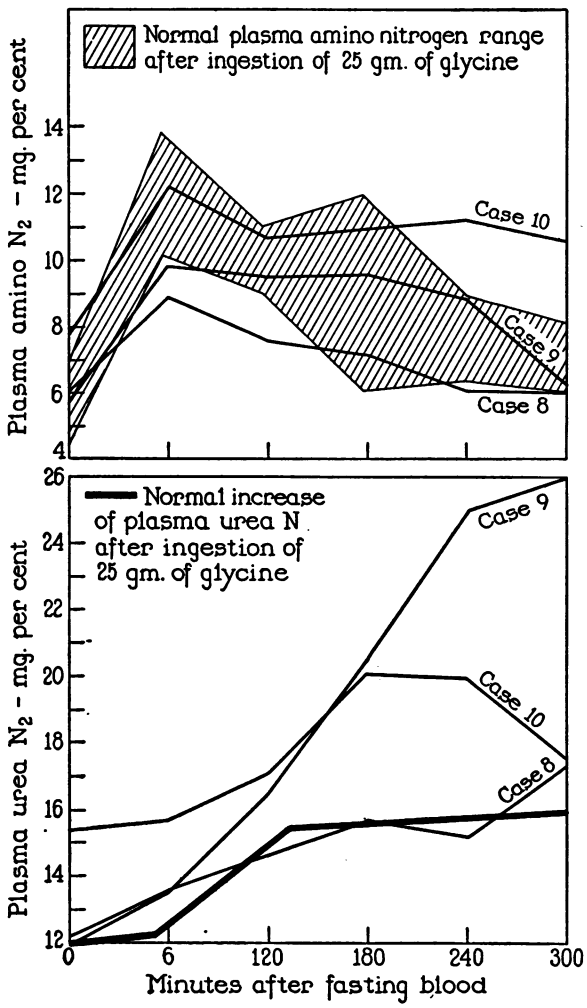

FIG. 4. PERNICIOUS ANEMIA, INDUCED REMISSIONS

was only 40 per cent of normal, and the elevated blood urea curves might be explained on the basis of renal insufficiency with retention.

5. Patients with refractory anemia (see Figure 5)

This group was composed of 3 patients (Cases 11, 12 and 13), 2 with aplastic anemia, and 1 with multiple myeloma, in whom the hemoglobin levels varied from 24 per cent to 42 per cent (see case histories). The plasma amino nitrogen curves were all within the normal range. The plasma urea nitrogen curves were above the normal, however, and in Case 13 the curve was markedly elevated. No explanation of this fact is at hand since the renal function was normal as indicated by the urea clearance. This observation should be investigated further.

6. Patients with ulcerative colitis (see Figure 6)

This group was composed of 4 patients (Cases 14, 15, 16, and 17) who for years had had persistent diarrhea with blood and pus in the stools. The glycine tolerance tests were made at a time when the symptoms were marked. The first portion of each of the plasma amino nitrogen curves was normal, but each had a delayed peak. In normal individuals the peaks occur about 60 minutes after glycine ingestion, but in the diarrheal

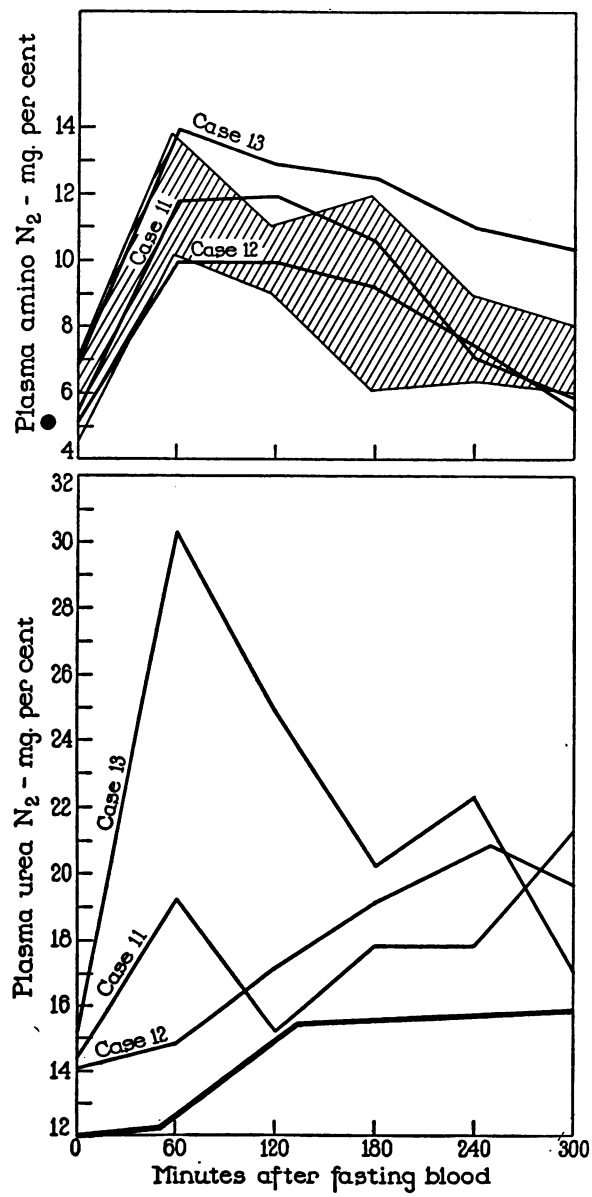

FIG. 5. REFRACTORY ANEMIA

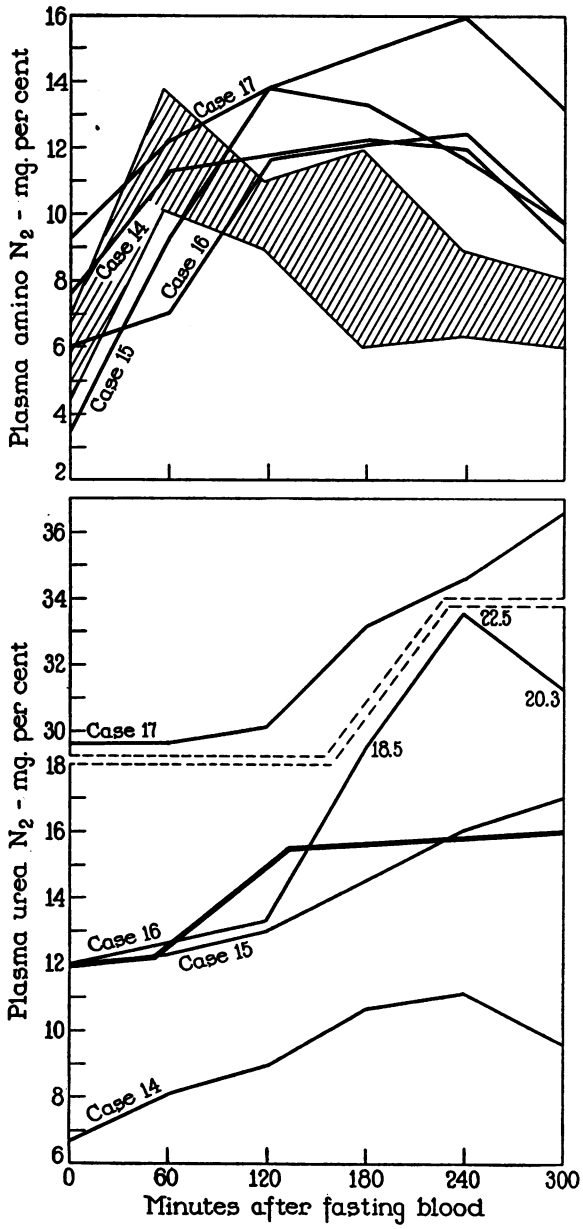

FIG. 6. ULCERATIVE COLITIS

patients they were delayed to between 120 and 240 minutes. The plasma urea nitrogen curves also had delayed peaks and 1 (Case 14) was below the normal level, whereas 2 (Cases 16 and 17) were definitely elevated. The urea clearance was 55 per cent of normal in Case 16 and 20 per cent of normal in Case 17. Presumably the elevations were due to renal insufficiency with retention of urea.

7. Patients with hepatic cirrhosis (see Figure 7)

This group was composed of 2 patients (Cases 18 and 19) who had clinical, laboratory, and finally postmortem evidence of advanced hepatic insufficiency with destruction of liver tissue. The plasma amino nitrogen curve in Case 18 had a delayed peak and in Case 19 it was flat but at a relatively high level. In both cases the plasma urea nitrogen curves were below normal.

DISCUSSION

Malabsorption of glucose and of fat has been established as a feature of tropical sprue, and evidence is available to suggest that glucose, at least, is not absorbed normally by patients with pernicious anemia. The results here presented indicate that the amino acid, glycine, also is poorly absorbed by patients with untreated sprue but after

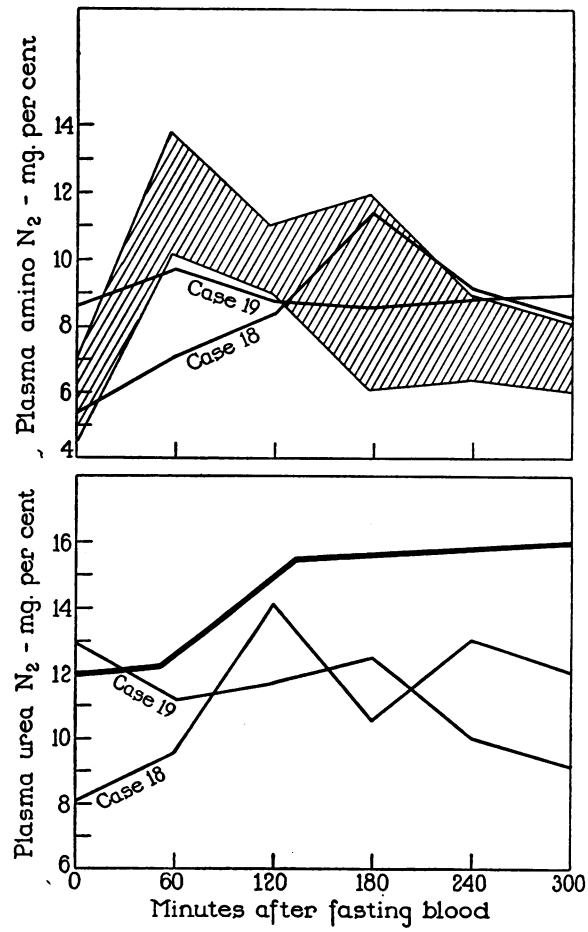

FIG. 7. HEPATIC CIRRHOSIS

the symptomatic improvement which follows the administration of liver extract the abnormality is no longer detectable. Furthermore, the low rates of formation and excretion of urea became normal after induced remission. The low curves for plasma amino and urea nitrogen observed before treatment can probably be attributed to poor absorption and not to simple loss of glycine from the bowel since the patients did not have diarrhea during the tests.

Once absorbed, carbohydrates, fats and proteins have not been shown to be abnormally metabolized in sprue; indeed, the results of glucose tolerance tests are normal even at the height of symptoms if the glucose is administered intravenously. It is possible, however, that the slow rates of urea formation before therapy by the patients here reported can be accounted for partially by some hepatic dysfunction in the metabolism of amino acids. In accord with this possibility is the fact that the results of 3 liver function tests were abnormal in both patients before treatment but became normal after the remissions were established.

In the 2 patients with pernicious anemia who had not been treated with liver extract previous to admission, the plasma amino nitrogen curves were also below normal and flattened. As in the cases with sprue, these results cannot be attributed to poor absorption because of diarrhea, for both patients had normal bowel function. Either there was poor absorption in both groups because of some specific dysfunction of the gastro-intestinal tract or the glycine was quickly deaminized. The latter assumption is unlikely since, if it were true, the rates of urea formation should be rapid, and this was not the case. An elevated plasma urea nitrogen was found in 1 case (Case 4), presumably due to urea retention, since only 1750 mgm. of urea were formed, and since the renal function test was but 28 per cent of normal. There was no clear evidence of hepatic dysfunction in this patient, although Fouts, Helmer, and Zerfas (14) have shown that the results of the sodium benzoate test are somewhat abnormal in patients with pernicious anemia. In the other patient (Case 3) the plasma urea nitrogen curve was below normal and 2714 mgm. of urea were formed; the urea clearance was 180 per cent and the rate of excretion of urinary urobilinogen was abnormally elevated (65.0 to 15.0 mgm. daily). In short, since no case of sprue or untreated pernicious anemia presented evidence of abnormally increased deamination and since no glycine was lost from the bowel during the test, it is assumed that the flat curves of plasma amino nitrogen indicate that absorption of glycine was impaired.

After therapy the curves became normal and by the same criteria it is assumed that the absorption of glycine improved. The fact that the amount of urea formed also became normal in both groups of patients is in accord with this assumption.

In the 3 tests made on patients with pernicious anemia in relapse following inadequate liver extract therapy, 1 plasma amino nitrogen curve was normal, and 2 were elevated. This fact indicates that the absorption of glycine was normal, but the rate of deaminization was possibly impaired in 2 cases. The results of renal and hepatic function tests were normal, this evidence being opposed to the presence of impaired hepatic deaminization or renal excretion as a cause of the high amino acid levels. The peaks of the plasma urea nitrogen curves were delayed in 2 patients, however, and in these the amount of urea formed was slightly less than normal (2774 mgm.) in one (Case 7) and much less than normal (1911 mgm.) in the other (Case 6). The peak of the curve was more delayed in the latter patient than in the former. The plasma urea nitrogen curve was normal in the third patient (Case 5) but the amount of urea formed was not measured. From the plasma urea nitrogen levels it appears that a subnormal rate of deaminization may be present in spite of good hepatic function, as indicated by other tests. After adequate treatment, the glycine curves became normal in all 3 patients.

The results suggest that relapse of previously treated pernicious anemia is attended by distinctly less disturbance of glycine absorption and metabolism than is shown by patients in their first attacks. It is noteworthy, however, that patients of the relapse group were tested when their anemia was much less severe than was that of either of the cases in their first attack, or of the patients with sprue.

The possibility that ischemia of the gastro-intestinal tract might play a rôle in the poor absorption of the amino acid by patients with low blood levels can be ruled out by the observation

made on 3 patients with severe refractory anemia. These had hemoglobin and erythrocyte levels as low as any of the patients with pernicious anemia or sprue, and yet their plasma amino nitrogen curves were essentially normal. Furthermore, the urea nitrogen curves were elevated in spite of normal urea clearances. Hence the absorption of glycine was probably even more rapid than the amino nitrogen levels would indicate.

Although, as previously mentioned, no patient had diarrhea during the period of the test it was still necessary to rule out more conclusively that symptom as a cause of malabsorption of glycine with resultant low curves of plasma amino nitrogen. Accordingly, the group of patients with ulcerative colitis and persistent active diarrhea was studied. In no instance was a low plasma amino nitrogen curve observed; indeed the levels were somewhat elevated, a fact supposedly due to a reduced rate of deaminization. This supposition could not be proved, however, since in Cases 16 and 17 high plasma urea values were found also, a fact referable to abnormally low rates of excretion (urea clearance 55 per cent and 20 per cent of normal, respectively). For the same reason, the subnormal sodium benzoate test results do not supply valid evidence of hepatic dysfunction as a possible cause of low deaminizing power.

In the studies already discussed, some evidence of hepatic dysfunction was at hand in those patients with sprue and untreated pernicious anemia who seemed to show evidence of poor absorption. No such evidence was found in the patients with pernicious anemia in relapse, and it was accordingly necessary to test the absorptive ability of patients with advanced hepatic insufficiency from cirrhosis. These were the only control studies which indicated a definite impairment of absorptive power. One plasma amino nitrogen curve was flat and below normal and the other was low, although a rise did appear late in the curve of the test. The urea nitrogen curves also were abnormally low. This would be expected if the defect were one of absorption. Definite evidence of hepatic dysfunction was at hand in the results of other tests on these patients.

It is suggested that the abnormal results of the glycine tolerance tests in patients with sprue and pernicious anemia in relapse are not dependent upon the associated diarrhea or anemia but possibly upon some hepatic dysfunction. The results of the glycine tolerance tests and hepatic function tests made on patients with hepatic cirrhosis were somewhat similar to those made on patients with untreated pernicious anemia and sprue. With adequate liver extract therapy the results of the tests in the latter groups of patients returned to normal or near normal levels but, as would be expected, this measure was ineffective in restoring normal hepatic function to patients in whom the majority of the liver cells had been replaced by fibrous tissue. The suggestion is also strong that abnormalities in the handling of glycine may be due to a lack of some constituent of liver extract, not the anti-pernicious anemia substance.

CONCLUSION

1. In 4 patients with untreated sprue and pernicious anemia the results of glycine tolerance tests suggested that glycine was absorbed from the gastro-intestinal tract more slowly than normal.

2. Evidence of this abnormality was not found in the same cases after the administration of liver extract.

3. Evidence of malabsorption was not demonstrable in patients with intractable diarrhea, severe refractory anemia or pernicious anemia in complete or partial remission, but was present in 2 patients with cirrhosis of the liver.

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