HEMATOLOGIC OBSERVATIONS IN PATIENTS WITH CHRONIC HEPATIC INSUFFICIENCY

STERNAL BONE MARROW MORPHOLOGY AND BONE MARROW PLASMACYTOSIS [1, 2]

By THOMAS JARROLD and RICHARD W. VILTER

(From the Department of Internal Medicine, College of Medicine, University of Cincinnati, Cincinnati)

(Received for publication June 9, 1948)

Macrocytic anemia is found in many persons with chronic hepatic disease. The incidence recorded by different observers had varied from 18% to 89%. Observations reported by Wintrobe (1) set the incidence of macrocytosis at 40.9% in 44 persons with cirrhosis and 62.5% in eight persons with cirrhosis and malignant disease of the liver. However, the pathogenesis of the anemia has not been clearly defined. In 1933 Wintrobe and Shumacker (2) and in 1936 Wintrobe (1) suggested that the anemia was due to the inability of a chronically and extensively diseased liver to store the antipernicious anemia factor. This opinion was supported by Goldhamer, Isaacs and Sturgis (3) who found that the antianemic substance was either reduced or absent in the livers of persons with hepatic cirrhosis and macrocytic anemia. However, in 1938 Schiff, Rich, and Simon (4) recovered an antipernicious anemia substance from the livers of cirrhotics with macrocytic anemia; they inferred that the defect was one of utilization rather than storage of this factor.

Poor hematologic response to liver extract has been the rule (1, 5), although an occasional patient with portal cirrhosis will respond as dramatically as a person with pernicious anemia in relapse (2); some cases are said to have responded to folic acid (6). In spite of these inconsistencies, the prevailing hypothesis still classes the macrocytic anemia of chronic liver disease with the erythrocyte maturation factor deficiency anemias. This is true even though the only report on bone marrow morphology failed to demonstrate megaloblastic maturation arrest (7). The data presented in this paper stress the normoblastic rather than megaloblastic type of bone marrow morphology found in the majority of patients with cirrhosis and macrocytic anemia. Thus any type of erythrocyte maturation factor defect becomes an unlikely etiologic possibility in these patients. Observations are also presented which correlate bone marrow plasmacytosis with hyperglobulinemia in persons with cirrhosis and suggest a causal relationship.

MATERIAL AND METHODS

The subjects chosen for study were 30 consecutive patients with a primary diagnosis of portal cirrhosis uncomplicated by recent hemorrhage. The diagnosis was made by history, physical examination, and by laboratory tests of liver function. Each of these tests was done at least two times on each patient and included the bromsulfalein retention, thymol turbidity, thymol flocculation,[3] cephalin-cholesterol flocculation,[3] quantitative serum bilirubin,[3] alkaline phosphatase,[3] and prothrombin time. The amount of urobilinogen in the stool [3] was determined in 11 cases. The serum albumin/globulin ratios were determined using a modified biuret method (8) and the Coleman Junior spectrophotometer. In 23 patients liver biopsies were done and the diagnosis was confirmed histologically.

The hematologic characteristics of each subject's peripheral blood were determined on two or more occasions. Reticulocyte counts were performed daily and erythrocyte and hemoglobin determinations were made every second day while the therapeutic effects of various agents were being investigated. Red and white blood cell counts were performed with pipettes and counting chambers certified by the U. S. Bureau of Standards. Hematocrit determinations were made on oxalated venous blood (4 mgm. of potassium oxalate and 6 mgm. ammonium oxalate per 5 cc. blood), centrifuged for 30 minutes in a Wintrobe tube at 3,000 r.p.m. Hemoglobin

[1] Presented in part before the American Society for Clinical Investigation, Atlantic City, N. J., May 3, 1948.

[2] This work has been supported by grants from the Robert Gould Research Foundation, Merck and Company, Inc., and the United States Public Health Service (RG 991). Lederle and Company supplied the Folic Acid.

[3] These tests were performed by the Gastric Laboratory of the Cincinnati General Hospital and the authors wish to acknowledge the cooperation of Dr. Leon Schiff, director of the Gastric Laboratory.

was determined as oxyhemoglobin with the Coleman Junior spectrophotometer. Reticulocyte and platelet counts were made by the wet technique using Dameshek's method (9). The cytology of the bone marrow obtained by sternal aspiration was studied one or more times. Cover slip preparations for cytologic study of capillary blood and sternal marrow were stained by Wright-Giemsa stains.

The differential counts of the sternal marrow were done independently by each of the authors. Agreement was excellent in each instance, and the average of the two counts was taken as the representative value. In each case 500 non-erythroid nucleated cells were counted. The erythroid nucleated cells were expressed as numbers per 100 non-erythroid nucleated cells.

During the period of hospitalization each of the patients was placed on a "hepatic regime." This included a high protein, high carbohydrate, high vitamin diet supplemented with crystalline B-complex vitamins, protein hydrolysates, ferrous sulfate, and such lipotropic substances as choline and methionine. Several patients were given crude liver extract ("Intraheptol") intravenously. In addition each patient was given crude or refined liver extract intramuscularly or folic acid orally. The crude liver extract was given in 5 cc. doses three times weekly and the refined liver in doses of 50 U.S.P. units three times each week. The folic acid was given in single oral doses of 100 mgm. daily. Patient No. 3 was kept on a vitamin B-poor diet and after ten days of observation with only an insignificant fall in erythrocytes was given one-half lb. of ground beef daily for three weeks. Thereafter this patient was treated in the manner described above.

RESULTS OF PERIPHERAL BLOOD STUDIES

Of the 30 persons studied, 24 were anemic, *i.e.*, they had less than 4,000,000 erythrocytes per cubic millimeter. Twenty of these had a macrocytic anemia (MCV of 96 cubic microns or above) and four had a normocytic anemia. The cells usually were normochromic. The remaining six patients were not anemic. Stained smears of the peripheral blood revealed moderate macrocytosis with minimal anisocytosis, poikilocytosis and polychromatophilia. Hypersegmented poly-

TABLE I

Initial examination of peripheral blood on 30 patients with portal cirrhosis

Case	Patient	Sex	Age	RBC (*millions*)	Hb. (*gms.*)	Reticulocytes (%)	Platelets	Hematocrit	MCV cu. μ	MCH $\gamma\gamma$	MCHC (%)	WBC	Differential WBC				
													Segmented neutrophiles	Lymphocytes	Monocytes	Eosinophiles	Basophiles
1	M. W.	F	36	1.71	6.6	7.7	212,040	19	111	38	34	10,450	76	15	9	0	0
2	C. G.	F	57	1.75	5.5	5.4	101,540	19	108	31	29	11,500	80	7	8	5	0
3	C. R. G.	F	29	1.82	8.3	6.0	650,000	25	137	45	32	11,400	73	15	9	2	1
4	R. P.	F	60	2.39	7.6	6.0	172,080	23	96	32	33	7,200	56	26	12	6	0
5	F. R.	F	34	2.82	10.2	3.4	327,100	33	113	35	31	4,400	80	12	4	2	2
6	G. K.	M	47	2.84	10.5	4.8	224,360	31	109	37	34	6,700	67	22	10	0	1
7	M. H.	F	43	2.87	8.6	3.8	201,480	25	90	30	34	7,200	70	15	8	6	1
8	E. M.	M	46	2.95	10.8	5.1	119,720	31	106	36	35	9,350	87	7	5	1	0
9	D. T.	F	17	2.95	8.0	6.1	259,600	25	84	27	32	11,280	90	3	4	2	1
10	C. N.	M	64	3.01	11.8	5.1	273,910	35	116	39	34	4,200	68	24	6	2	0
11	W. J.	F	40	3.04	10.8	3.8	346,560	34	108	35	32	5,750	73	18	8	0	1
12	F. H.	M	65	3.10	9.8	2.7	285,200	30	96	31	33	5,250	67	29	4	0	0
13	C. K.	M	55	3.10	9.8	4.5	328,600	32	103	32	31	9,100	86	6	8	0	0
14	T. R.	M	43	3.30	12.0	1.7	233,220	37	109	36	32	8,550	78	10	12	0	0
15	R. T.	M	37	3.40	11.3	1.6	373,680	39	112	33	29	6,450	87	8	5	0	0
16	E. N.	M	33	3.56	11.3	7.5	512,640	35	98	32	32	8,650	72	23	4	0	1
17	H. R.	M	58	3.59	14.0	4.6	247,710	41	114	39	34	19,000	93	4	3	0	0
18	E. M.	M	40	3.63	13.1	1.4	268,620	37	101	36	35	5,600	64	26	8	1	1
19	F. A.	F	68	3.67	12.6	5.1	242,000	39	108	34	32	11,200	76	17	4	0	3
20	E. P.	F	51	3.77	14.0	4.6	418,470	40	106	37	35	7,450	64	23	12	0	1
21	L. D.	M	46	3.81	11.3	6.7	262,890	34	89	30	33	4,500	76	13	11	0	0
22	A. S.	M	31	3.81	12.6	3.3	251,460	39	102	33	32	4,250	56	27	12	4	1
23	L. B.	M	57	3.88	12.6	2.5	492,760	39	100	32	32	6,100	54	34	8	3	1
24	J. F.	M	59	3.90	11.3	1.4	245,552	36	90	29	31	3,700	56	27	15	1	1
25	A. M.	F	32	4.02	13.3	4.5	249,240	40	99	33	33	9,200	79	11	4	2	4
26	C. R. K.	M	50	4.07	12.5	13.5	390,372	38	93	31	33	5,450	66	19	11	2	2
27	G. F.	M	70	4.07	13.3	2.7	305,250	40	98	33	33	5,200	58	32	10	0	0
28	C. K.	M	77	4.17	13.7	2.5	362,790	43	103	33	32	5,150	62	30	6	1	1
29	H. S.	M	47	4.17	14.0	2.7	487,890	41	98	34	34	6,000	66	28	5	1	0
30	W. R.	M	59	5.33	15.3	2.0	373,100	48	90	29	32	10,900	68	24	6	1	1

TABLE II

Differential bone marrow cell counts in 30 subjects with portal cirrhosis

Case	Patient	Polymorphonuclear leucocytes (%)	Metamyelocytes (%)	Myelocytes "C" (%)	Myelocytes "B" (%)	Myelocytes "A" (%)	Myeloblasts (%)	Lymphocytes (%)	Monocytes (%)	Eosinophiles (%)	Eosinophilic myelocytes (%)	Basophiles (%)	Plasmacytes (%)	Other cells (%)	Megaloblasts per 100 WBC	Early erythroblasts per 100 WBC	Late erythroblasts per 100 WBC	Normoblasts per 100 WBC	Number of nucleated RBC per 100 WBC
1	M. W.	40.0	14.0	6.5	3.5	1.0	0	15.0	0	0.5	0.5	0	19.0	0	0.5	1.5	7.0	41.0	50.0
2	C. G.	31.5	11.5	9.0	3.5	3.5	0.5	10.5	0.5	8.0	3.0	1.0	16.0	1.5	0	1.0	7.0	76.5	84.5
3	C. R. G.	38.5	30.0	10.5	2.5	1.5	0.5	9.5	0.5	3.5	0.5	0	2.0	0.5	1.0	3.5	12.0	30.0	46.5
4	R. P.	40.5	14.5	12.0	5.0	2.0	0	10.0	0.5	2.5	0.5	0.5	7.5	4.5	0	0.5	2.0	21.0	23.5
5	F. R.	45.0	12.0	9.0	7.0	1.5	0.5	10.5	0	3.5	0.5	0	7.0	3.5	2.0	3.0	8.0	36.0	49.0
6	G. K.	41.5	13.0	10.5	5.5	0.5	0.5	9.5	1.5	3.0	0.5	0	9.5	4.5	0	1.5	3.5	54.0	59.0
7	M. H.	38.5	21.5	10.5	3.0	2.5	0	5.0	0.5	0.5	2.0	0	14.0	2.0	0	1.0	4.0	40.0	45.0
8	E. M.	25.0	14.0	7.5	4.5	5.5	2.5	13.0	1.5	3.0	0.5	0.5	13.0	9.5	0	2.0	8.5	118.0	128.5
9	D. T.	43.0	16.0	8.0	8.0	1.0	0.5	12.0	0.5	1.0	1.0	1.0	7.0	1.0	0	5.0	15.0	75.0	95.0
10	C. N.	39.5	16.0	6.5	4.5	1.5	0	11.5	0	3.5	1.0	0	10.0	6.0	0	1.5	11.0	76.0	88.5
11	W. J.	28.5	30.0	6.5	2.5	1.0	1.5	7.5	1.0	0.5	3.5	1.0	13.5	3.0	0	1.5	6.5	34.5	42.5
12	F. H.	32.5	21.5	7.5	1.5	2.5	1.5	12.5	0	7.0	3.5	1.5	6.5	2.0	0	0.5	1.0	26.0	27.5
13	C. K.	39.5	19.0	9.5	2.5	1.5	0	5.0	0.5	4.0	2.0	1.0	12.0	3.5	0	1.0	5.5	32.5	39.0
14	T. R.	35.0	21.0	9.0	3.5	2.5	1.5	16.0	0	1.5	0	0	7.0	3.0	3.0	12.0	28.0	48.0	91.0
15	R. T.	52.0	14.5	7.5	2.5	1.0	1.0	6.0	0.5	4.5	1.0	0	3.5	6.0	0.5	3.0	10.0	64.5	78.0
16	E. N.	29.5	18.5	4.5	2.0	1.5	1.0	10.5	0.5	1.0	2.0	0	23.5	5.5	0.5	3.0	5.0	65.0	73.5
17	H. R.	29.5	12.5	13.0	7.5	1.0	0.5	4.0	0.5	5.5	0.5	0.5	20.0	5.0	0	2.5	10.5	56.5	69.5
18	E. M.	43.5	15.0	6.0	3.0	1.5	1.5	14.5	1.0	1.0	1.0	2.0	8.0	2.0	0	1.0	4.5	32.0	37.5
19	F. A.	38.0	11.5	4.0	6.5	2.0	1.5	13.0	2.0	6.0	2.0	1.0	9.5	3.0	0	2.0	3.0	29.0	34.0
20	E. P.	34.0	16.5	10.0	3.5	1.0	0	9.5	0	5.5	1.5	0	14.0	4.5	0	3.0	10.5	96.0	109.5
21	L. D.	49.5	15.5	3.5	2.0	2.5	0.5	5.5	0	7.5	3.5	0	9.0	1.0	0	1.0	4.0	52.5	57.5
22	A. S.	37.5	11.5	9.0	3.0	1.0	1.0	13.5	0.5	1.5	6.5	0.5	10.5	4.0	0.5	1.5	9.0	83.5	94.5
23	L. B.	43.5	11.0	6.5	1.0	3.5	0	10.5	0.5	6.0	2.5	1.0	11.0	3.0	0	0.5	2.0	41.0	43.5
24	J. F.	45.5	21.5	10.0	3.0	1.0	0	7.5	0.5	4.0	1.0	0.5	2.5	3.0	0	1.0	5.0	15.0	21.0
25	A. M.	36.5	13.0	6.0	1.5	3.0	3.0	19.0	1.0	2.0	0.5	0.5	8.0	6.0	0	1.0	7.0	76.5	84.5
26	C. R. K.	17.5	14.0	18.0	9.0	4.0	0.5	15.0	1.0	5.0	3.0	0.5	6.0	6.5	0.5	3.0	5.0	58.0	66.5
27	G. F.	34.0	30.0	7.0	2.0	0.5	0.5	14.0	0.5	3.0	1.0	0	6.5	1.0	0	1.5	3.5	54.0	59.0
28	C. K.	27.0	13.0	5.0	5.0	3.0	0	19.0	0	5.0	2.0	2.0	15.0	4.0	1.0	3.0	9.5	78.0	91.5
29	H. S.	48.5	13.5	3.5	1.5	3.0	0	8.0	0.5	4.5	1.5	0	8.5	7.0	0	1.0	2.5	66.0	69.5
30	W. R.	32.5	16.0	8.0	4.5	0	1.5	10.5	0.5	3.5	1.0	0	15.5	6.5	0.5	1.5	7.0	41.0	50.0

morphonuclear leucocytes were not observed. Reticulocytosis, usually ranging from 2% to 7.7% but in one instance as high as 13.5%, was found even though these patients did not show evidence of recent hemorrhage. The guaiac test for occult blood in the stool was faintly positive in several of these patients; but the degree of reticulocytosis was correlated with an increased bilirubinemia much more closely than with such minimal evidence of bleeding. The hematologic characteristics found in these 30 patients with portal cirrhosis are recorded in Table I.

RESULTS OF BONE MARROW STUDIES

The bone marrow appeared normally cellular in 21 subjects and moderately hypocellular in nine. The cytologic aspects and distribution of nucleated cells of the erythroid series usually were normal, although in 11 subjects there was moderate hyperplasia at the normoblast level (see Table II). Such hyperplasia was found in the most anemic patients. In only three patients (Nos. 3, 5, and 14) was there a suggestion of the "megaloblastic maturation arrest" so characteristic of pernicious anemia and related macrocytic anemias. These patients were remarkable in that they were the only ones with signs of advanced multiple B-complex vitamin deficiencies, and each had a dietary history consistent with a prolonged deficiency of extrinsic factor. One of these patients (Case No. 5) had 57° free hydrochloric acid in the gastric secretions following histamine stimulation. The other two had none. Unfortunately there was no opportunity to repeat the test in the latter cases.

The granulocytic cells were normal; there were no bizarre metamyelocytes. The lymphocyte percentages were at the upper limits of normal.

There was, however, a striking and consistent increase in the number of plasmacytes. In the 30 patients the average was 10.5% with extremes of 2% to 23.5%. Normally, in our laboratory plasmacytes recorded as a percentage of nucleated nonerythroid cells range from 0 to 3.5% with an average of 1%. This plasmacyte hyperplasia was approximately proportional to the degree of hyperglobulinemia found in these patients. Table III and Figure 1 illustrate this relationship. In addition to the 30 patients with portal cirrhosis, we have studied the bone marrow of seven persons with biliary cirrhosis and seven with infectious hepatitis as revealed by liver biopsies. In these 14 cases, unlike those with portal cirrhosis, plasmacytosis as high as 7% occurred only once and in none was there any correlation between the number of plasmacytes and the amount of serum globulin.

TABLE III

Correlation between serum globulin and plasmacytosis in the bone marrows of 30 patients with cirrhosis

No.	Pt.	Serum protein *gms./100 cc.*			Average globulin	Bone marrow plasmacytes	Average plasmacytes
		Total	Albumin	Globulin			
					gms./100 cc.	*per cent*	*per cent*
3	C. R. G.	6.2	4.0	2.2		2.0	
24	J. F.	5.7	3.5	2.2		2.5	
12	F. H.	5.7	2.9	2.8	2.60	6.5	4.1%
15	R. T.	6.0	3.1	2.9		3.5	
26	C. R. K.	5.7	2.8	2.9		6.0	
9	D. T.	5.7	2.6	3.1		7.0	
25	A. M.	7.5	4.3	3.2		8.0	
10	C. N.	5.2	2.0	3.2	3.22	10.0	8.0%
5	F. R.	6.1	2.8	3.3		7.0	
18	E. M.	6.8	3.5	3.3		8.0	
27	G. F.	5.7	2.0	3.4		6.5	
19	F. A.	5.6	2.2	3.4		9.5	
14	T. R.	6.9	3.5	3.4	3.46	7.0	9.1%
6	G. K.	6.4	2.8	3.5		9.5	
8	E. M.	5.3	1.7	3.6		13.0	
30	W. R.	5.1	1.5	3.6		15.5	
29	H. S.	6.3	2.5	3.8		8.5	
21	L. D.	5.8	2.0	3.8	3.82	9.0	12.0%
23	L. B.	7.7	3.9	3.8		11.0	
2	C. G.	6.1	2.0	4.1		16.0	
16	E. N.	6.7	2.6	4.1		23.5	
28	C. K.	6.0	1.8	4.2		15.0	
13	C. K.	6.6	2.3	4.3	4.28	12.0	13 7%
4	R. P.	6.7	2.3	4.4		7.5	
22	A. S.	7.6	3.2	4.4		10.5	
7	M. H.	7.2	2.7	4.5		14.0	
20	E. P.	6.7	2.0	4.7		14.0	
17	H. R.	6.2	1.5	4.7	4.72	20.0	16.1%
1	M. W.	6.4	1.6	4.8		19.0	
11	W. J.	9.1	4.2	4.9		13.5	

The cases are arranged in the order of increasing globulin values. Under these conditions there is a similar progressive increase in the percentage of plasma cells.

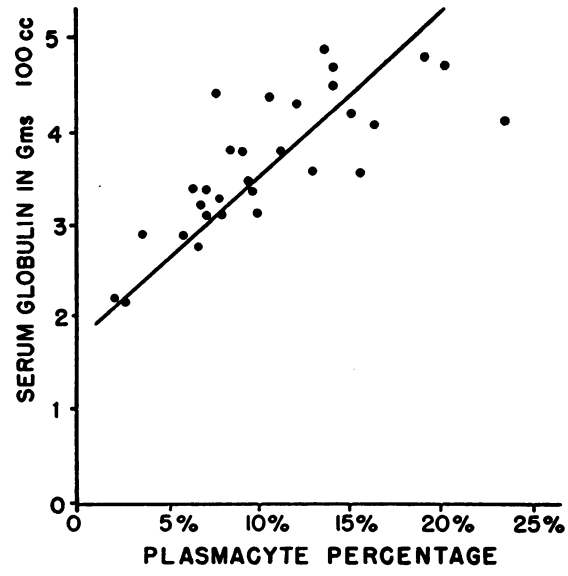

FIG. 1. GRAPHIC ILLUSTRATION OF DATA PRESENTED IN TABLE III

PROGRESS AND RESPONSE TO TREATMENT

There was no evidence of blood regeneration in the majority of patients on the hepatic regime supplemented with either liver extract or folic acid as previously described (see Figure 2). Slow non-specific hematologic improvement was observed in only five patients. This coincided with improvement in the patient's general condition.

A specific hematologic response similar to that which follows the administration of liver extract to persons with pernicious anemia was observed in only two patients (Nos. 3 and 14). A third patient (No. 5) who received one-half lb. of beef daily for 21 days in place of the hepatic regime, had an erythrocyte and hemoglobin rise of 2.75 to 3.72 millions and 10.3 to 12.2 grams respectively in this period of time. The reticulocytes did not increase during this period but were sustained between 4% and 5%, gradually falling toward the end of the period. The mean corpuscular volume fell from 120 to 108 cubic microns. Each of these three patients (Nos. 3, 5, and 14) had a significant number of megalo-

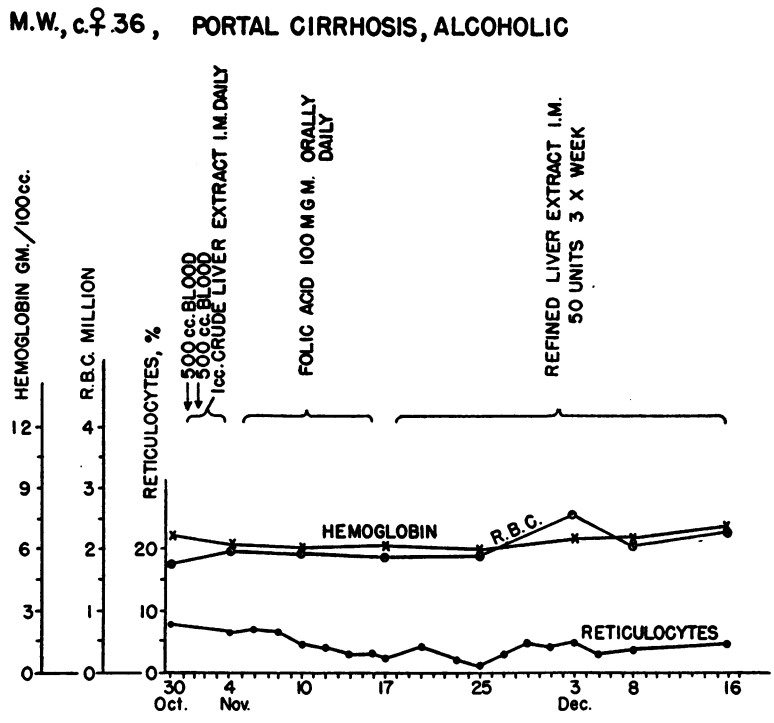

FIG. 2. THIS PATIENT HAD PROVED PORTAL CIRRHOSIS AND A MACROCYTIC ANEMIA

This graph illustrates the ineffectiveness of liver extract and folic acid in influencing the course of this anemia.

blasts and early erythroblasts in the bone marrow, but patients Nos. 3 and 14 were too ill to be subjected to the same experiment as patient No. 5. In fact patient No. 14 deteriorated rapidly even though there had been a hematologic response, and expired three weeks after hospital admission. Wintrobe (1) has recorded a similar experience.

DISCUSSION

The characteristics of the peripheral blood of the cirrhotic subjects in this study are comparable to those previously described (1, 5). Sixty-five per cent had macrocytic anemia; 15% had normocytic anemia and 20% no anemia. The accumulated evidence indicates that in the great majority of these subjects the macrocytic anemia of chronic liver disease occurred because of a metabolic defect other than erythrocyte maturation factor (liver factor) deficiency. The peripheral blood of persons with portal cirrhosis and macrocytic anemia did not reveal the anisocytosis, poikilocytosis, nucleated erythrocytes or multilobed polymorphonuclear leucocytes characteristic of pernicious anemia and related diseases. The bone marrow was normoblastic and either normally cellular or slightly hypocellular rather than hypercellular and megaloblastic. Finally, there was no change in the bone marrow or peripheral blood of persons with uncomplicated cirrhosis which could be credited to treatment with crude or refined liver extract or folic acid in large doses.

Three patients in this series had eaten diets for many years which were grossly deficient in B-complex vitamins, animal protein and presumably extrinsic factor. These were the only persons who exhibited advanced lesions of vitamin B-complex deficiency disease, such as glossitis, neuritis, and pellagrous dermatitis; these were the instances in which there were a significant number of megaloblasts or early erythroblasts in the bone marrow and in which there was a hematologic response to liver extract, and in one case, a response following the administration of ground beef. We believe that these three patients and the several similar patients reported in the medi-

cal literature (1, 10) are examples of extrinsic factor deficiency macrocytic anemia in persons with cirrhosis rather than the macrocytic anemia of chronic liver disease.

Several other points warrant brief comment. Blood loss cannot be invoked to explain the consistent initial reticulocytosis found in these patients and described previously by Rosenberg (5). Hemolysis or mild hypersplenism are unlikely causes because the level of urobilinogen in the stools was not elevated in any patient in whom the test was performed, and the bilirubin in the blood was usually of the direct reacting type. The uniform type of macrocytosis cannot be explained satisfactorily. Both of these problems require further investigation.

The bone marrow plasmacytosis in portal cirrhosis, which correlated roughly with the degree of hyperglobulinemia, has its counterpart in several other diseases and has been noted previously in European communications (11, 12). Such a correlation has also been found in lymphopathia venereum, Boeck's sarcoid, kala azar, multiple myeloma and serum sickness (13–17). As a result of studies on diseases associated with elevated serum globulin, Bing and Plum (18) conclude that the formation of globulin takes place in the plasmacyte. This opinion is supported by more recent investigations concerning the relationship of plasmacytosis to hyperimmune reactions and hyperglobulinemia (17–22). These studies indicate that plasmacytes develop in bone marrow and spleen in animals sensitized to bacterial or simple protein antigens as antibody globulin increases. Passively induced hyperglobulinemia or passive sensitization and shock do not induce a plasmacyte response.

Our studies do not indicate whether globulin is formed by plasmacytes or whether the plasmacytes appear in response to globulin formed elsewhere. Because of the studies cited above and by analogy with multiple myeloma it is likely that the plasmacyte is responsible for the excessive production of one of the beta or gamma globulins found by electrophoresis in the serum of patients with myeloma or portal cirrhosis (23). The stimulus to plasmacytosis in cirrhosis may be an antigen which the damaged liver either liberates or cannot inactivate.

Other globulins may be produced by lymphocytes and by the macrophages of the bone marrow, liver and other parts of the reticulo-endothelium without the mediation of the plasmacyte. Perhaps this is the reason why globulin may be elevated in acute hepatitis and biliary cirrhosis without a consistent increase in plasmacytes in bone marrow or other tissues.

CONCLUSIONS

1. A hematological survey has been carried out in 30 patients with proved portal cirrhosis.
2. Moderate or severe macrocytic anemia was present in 65% of the patients.
3. Reticulocytosis usually ranged up to 8% but in one instance was as high as 13.5%. It could not be explained by acute loss of blood.
4. The bone marrow of these persons was normally cellular or moderately hypocellular. In 11 there was moderate normoblastic hyperplasia. The only patients with megaloblastic maturation arrest had evidence of "extrinsic factor" deficiency.
5. Plasmacytes were consistently increased in the sternal bone marrows of these patients. The degree of plasmacytosis correlated roughly with the degree of hyperglobulinemia, suggesting a cause and effect relationship of a similar nature to that observed in multiple myeloma.
6. The macrocytic anemia failed to respond to liver extract and folic acid although there was an occasional spontaneous hematologic remission associated with a decrease in the severity of the cirrhosis. There was a significant hematologic response to liver extract or ground beef only in the three patients in whom extrinsic factor deficiency was suspected.
7. The normoblastic bone marrow and the lack of response to liver extract and folic acid in the majority of cases strongly suggests that the macrocytic anemia of chronic liver disease is produced by a metabolic defect entirely different from that responsible for pernicious and related macrocytic anemias.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Miss Virginia R. Hawkins in performing the hematologic determinations of the peripheral blood and Mrs. Betty Fichter in carrying out the serum albumin-globulin determinations. We also wish to acknowledge the assistance

rendered by Dr. Robert Cogswell who performed the liver biopsies and to Dr. Daniel Richfield who examined the histologic specimens.

BIBLIOGRAPHY

1. Wintrobe, M. M., Relation of disease of liver to anemia. Arch. Int. Med., 1936, **57**, 289.
2. Wintrobe, M. M., and Shumacker, H. S., Jr., Occurrence of macrocytic anemia in association with disorder of liver, together with consideration of relation of this anemia to pernicious anemia. Bull. Johns Hopkins Hosp., 1933, **52**, 387.
3. Goldhamer, S. M., Isaacs, R., and Sturgis, C. S., The role of the liver in hematopoiesis. Am. J. M. Sc., 1934, **188**, 193.
4. Schiff, L., Rich, M. L., and Simon, S. D., "Hæmatopoietic principle" in diseased human liver. Am. J. M. Sc., 1938, **196**, 313.
5. Rosenberg, D. H., Macrocytic anemia in liver disease, particularly cirrhosis. Am. J. M. Sc., 1936, **192**, 86.
6. Spies, T. D., Experiences with Folic Acid. Yearbook Publishers, Inc., Chicago, 1947.
7. Limarzi, L. R., Jones, R. M., Paul, J. T., and Poncher, H. G., Sternal marrow in Banti's syndrome and other splenomegalic states. Am. J. Clin. Path., 1943, **13**, 231.
8. Robinson, H. W., and Hogden, C. G., Biuret reaction in determination of serum proteins. J. Biol. Chem., 1940, **135**, 707.
9. Dameshek, W., A method for the simultaneous enumeration of blood platelets and reticulocytes, with consideration of normal blood platelet count in men and in women. Arch. Int. Med., 1932, **50**, 579.
10. Wayburn, E., Macrocytic anemia in liver disease. California & West. Med., 1942, **56**, 130.
11. Rohr, K., Blut- und Knochenmarksmorphologie der Agranulocytosen. Folia Haemat., 1936, **55**, 305.
12. Fleischhacker, H., Über die Plasmazellen und das reticuloendotheliale System des Knochenmarkes. Beitrag zur Herkunft der Plasmaeiweisskörper. Deutsches Arch. f. klin. Med., 1940, **186**, 506.
13. Fleischhacker, H., Über die Bedeutung der Reticuloendothelien und Plasmazellen des Knochenmarkes. Ergebn. d. inn. Med. u. Kinderh., 1941, **60**, 508.
14. Kagan, B. M., Hyperglobulinemia. Am. J. M. Sc., 143, **206**, 309.
15. Taussig, A. E., and Somogyi, M., Hyperglobulinemia in granuloma inguinale. J. Lab. & Clin. Med., 1940, **25**, 1070.
16. Ranstrom, S., On the terms "essential hyperglobulinemia" and "premyeloma." Acta med. Scandinav., 1946, **124**, 134.
17. Gormsen, H., and Heintzelmann, F., Behavior of sedimentation reaction, serum proteins and sternal punctate in serum sickness. Nord. Med. (Hospitalstid), 1941, 11, 2125.
18. Bing, J., Further investigations on hyperglobulinemia. Acta med. Scandinav., 1940, **103**, 565.
19. Kolouch, F., Good, R. A., and Campbell, B., The reticuloendothelial origin of the bone marrow plasma cells in hypersensitive states. J. Lab. & Clin. Med., 1947, 32, 749.
20. Bjørneboe, M., and Gormsen, H., Experimental studies on the role of plasma cells as antibody producers. Acta path. & microbiol. Scandinav., 1943, **20**, 649.
21. Bjørneboe, M., Gormsen, H., and Lundquist, F. A., Further experimental studies on the role of the plasma cells as antibody producers. J. Immunol., 1947, **55**, 121.
22. Good, R. A., Effect of passive sensitization and anaphylactic shock on rabbit bone marrow. Proc. Soc. Exp. Biol. & Med., 1948, **67**, 203.
23. Gray, S. J., and Barron, E. S. G., Electrophoretic analyses of serum proteins in diseases of liver. J. Clin. Invest., 1943, 22, 191.