higher proportion of patients with circulating antibodies to extracts of colonic mucosa(4). The indirect Coon's technic, however, facilitates the study of non-precipitating antibodies and the localization and histochemical properties of antigenic material in the colon.

The serum globulin binding to hepatic ductules may indicate that common or similar cross-reacting antigens reside in colon and liver. A common embryological association of these organs and the known propensity for hepatic damage in ulcerative colitis(14) support this hypothesis.

The demonstration of antibodies to colon mucosa and the definition of their respective antigens form the basis for further study of immunologic processes occurring in ulcerative colitis. Future investigation may determine the pathogenetic significance of these substances.

Conclusion. Increased gamma globulin was demonstrated in the intestinal tract of patients with active regional ileitis and ulcerative colitis. The indirect fluorescent antibody technic revealed a gamma globulin in the serum of 3 of 25 patients with ulcerative colitis which was bound to epithelial glands of colonic mucosa and of hepatic bile ductules. Sera of normals and hospital controls did not bind to colonic mucosa.

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Quantitative Chromatographic Analysis of the Phospholipids of Abnormal Human Red Blood Cells.* (27203)

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The abnormal shape of the red blood cells in patients with hereditary spherocytosis, pernicious anemia, sprue, and thalassemia raises the possibility of a defect in the red blood cell membrane as a basis for the red blood abnormality. Since almost all of the lipid of the red blood cell resides in the membrane (1), a study of the red blood cell lipid offers one approach to this problem. Indeed, Williams *et al.*(2,3), in a study of the red blood cells of patients with pernicious anemia, observed a decreased concentration of lipid phosphorus and free cholesterol, an increased concentration of cholesterol ester, and development of an abnormal distribution of the individual phospholipids with treatment. Moreover, Allison *et al.*(4) and Kates *et al.* (5), studying the red blood cells of 2 patients with hereditary spherocytosis, found an in-

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crease in the relative concentration of lysophosphatidyl ethanolamine and a decrease in the relative concentration of phosphatidyl ethanolamine and postulated that a defect in conversion of the former to the latter was the basis for the spherocytosis.

In a previous study(6), the red blood cell phospholipids of normal subjects were separated by chromatography on silicic acid columns into "cephalin", lecithin, sphingomyelin, and lysolecithin fractions and the concentration of each determined. In the present study, the same technic was used to analyze the red blood cell phospholipids of patients with hereditary spherocytosis, pernicious anemia, sprue, intermediate thalassemia, sickle cell anemia, and polycythemia vera.

Materials and methods. The red blood cells of 14 patients were studied. The 3 patients with hereditary spherocytosis, all of whom had had splenectomies, had normal hemoglobin levels. In Patient S.G., the red blood cell analysis was performed 8 days after splenectomy. Patients M.K. and C.F., with untreated typical pernicious anemia, had hematocrits of 25 and 34, respectively, while the 2 patients with treated pernicious anemia, H.S. and A.H., had normal hemoglobin levels. Patient S.D., with non-tropical sprue, had a macrocytic anemia with a hematocrit of 27. Patient C.G. was a negro with a congenital hemolytic anemia which was thought to be best classified as an intermediate thalassemia; 40% of his hemoglobin was of the fetal type, but no other hemoglobin abnormality was evident. He had hepatosplenomegaly, leg ulcers, increased red blood cell resistance to osmotic lysis, about 50% target cells, and a hematocrit of 25. The 3 patients with sickle cell anemia, R.P., C.Wa., and M.S., had hematocrits of 29, 26, and 33, respectively. Patients C.J., and L.A., with polycythemia vera, had hematocrits of 48 and 85, respectively; the hematocrit of the former patient had been lowered to this level by phlebotomy prior to study.

The blood was taken in the fasting state in all of the patients except F.G., H.S., and A.H. Heparin was used for anticoagulation in all of the samples except in Patients F.G., H.S., A.H., and C.J., where the blood was defibrinated. The red blood cells were washed 3 times with isotonic saline and extracted with chloroform-methanol (2:1); the extracts, after storage for variable periods *in vacuo* at −30°C, were chromatographed on silicic acid columns using chloroform-methanol-water mixtures for elution. The methods used for extraction and chromatography have been described(6,7). The chromatographic analysis of each sample was done in duplicate. The series of control values used for red blood cell phospholipids (Table I) consisted of a published series of 6 normal fasting subjects (6) and an unpublished series of 7 normal non-fasting subjects; no difference between red blood cell phospholipid concentrations measured fasting and non-fasting was demonstrable.

Results. The percentage distributions and concentrations of the individual red blood cell phospholipids of the patients studied are shown in Table I. No attempt was made to analyze the "cephalin" fraction for its components, which included ethanolamine-, serine-, and inositol-containing phospholipids (6). On the basis of the 4 fractions analyzed, no consistent difference from normal in the percentage distribution of the individual phospholipid fractions was apparent. In Patient S.G., with hereditary spherocytosis, the relative concentration of "cephalin" and probably lecithin was decreased and sphingomyelin increased. Variations in the absolute concentrations of the individual phospholipid fractions reflected differences in total red blood cell lipid phosphorus concentration. The total lipid phosphorus concentration in the patients with intermediate thalassemia and sickle cell anemia was increased while in the patients with untreated pernicious anemia and sprue this value may have been slightly decreased.

Since Allison *et al.*(4) and Kates *et al.*(5) reported an increase in concentration of lysophosphatidyl ethanolamine in the red blood cells of patients with hereditary spherocytosis, lysophosphatidyl ethanolamine was prepared by the action of snake venom (*Naja Naja*) on an emulsion of egg "cephalin," which is composed predominantly of phosphatidyl ethanolamine(8), in order to study

TABLE I. Phospholipid Composition of Abnormal Human Red Blood Cells in 14 Patients.

Patient	Diagnosis	Sex	Age	Phospholipids				Recovery	Total lipid P, mmoles/l
				"Cephalin"	Lecithin	Sphingomyelin	Lysolecithin		
				%					
	Normals (13)		Mean	42.4	32.7	23.1	1.8	97	4.50
			S.D.	1.0	2.0	1.9	.2	3	.36
C.W.	Hereditary spherocytosis	♀	29	42.4	32.6	23.3	1.7	99	4.08
F.G.	*Idem*	♀	16	41.4	33.3	23.2	2.1	97	4.75
S.G.	"	♀	23	39.5	29.3	29.2	2.0	100	3.97
M.K.	Pernicious anemia	♀	61	42.9	33.9	21.4	1.8	98	3.82
C.F.	*Idem*	♂	48	40.1	37.6	20.6	1.7	94	4.25
H.S.	" treated	♂	72	42.3	34.9	20.9	1.9	97	4.84
A.H.	" "	♀	70	44.1	32.3	21.9	1.7	102	4.54
S.D.	Sprue	♂	61	43.7	34.8	19.8	1.7	95	3.94
C.G.	Intermediate thalassemia	♂	35	43.0	33.9	21.3	1.8	97	6.56
R.P.	Sickle cell anemia	♂	13	44.4	29.7	23.8	2.1	95	6.34
C.Wa.	*Idem*	♀	21	44.9	35.0	18.6	1.5	97	5.78
M.S.	"	♂	18	42.1	32.9	23.5	1.5	84	5.21
L.A.	Polycythemia vera	♂	14	40.9	35.3	21.8	2.0	93	4.93
C.J.	*Idem*	♀	59	40.1	33.7	23.9	2.3	93	5.08

its chromatographic behavior. When this lysophosphatidyl ethanolamine was chromatographed in the system used in the present study, it was eluted with the lecithin fraction; when mixed with a lipid extract of normal human red blood cells and chromatographed in the same way, it was again eluted with the lecithin fraction. When chromatographed on silicic acid-impregnated paper with 20% methanol in chloroform (v/v), it had an R_f similar to that of sphingomyelin.

Discussion. In a previous study(6), the phospholipids of normal human red blood cells were separated into "cephalin," lecithin, sphingomyelin and lysolecithin fractions by chromatography on silicic acid columns and the concentration of each fraction was estimated. These findings were confirmed by Reed *et al.*(9), who, in a more detailed analysis using chromatography on silicic acid-impregnated paper, also measured the concentrations of the constituents of the "cephalin" fraction, *i.e.*, ethanolamine-, serine-, and inositol-containing phospholipid, as well as of polyglycerol phosphate. In the present study, in which only the 4 fractions separated on silicic acid column chromatography were measured, no consistent abnormality in distribution of these fractions was found in patients with hereditary spherocytosis, pernicious anemia, sprue, intermediate thalassemia, sickle cell anemia, and polycythemia vera. In the patients with sickle cell anemia, where a hemoglobin defect has been demonstrated(10), and with polycythemia vera, where no red blood cell abnormality has been shown, no abnormality in the red blood cell phospholipids was expected. In the other patients, however, in whom the basic red blood cell defect is not known, the abnormal shapes of these cells raise the possibility of a defect in the cell membrane, where almost all of the red blood cell lipid resides(1). The present study, however, indicates that an abnormality in distribution of the major phospholipid fractions did not account for the red blood cell defects of the patients studied.

These findings, however, are contrary to the results of others. Allison *et al.*(4) and Kates *et al.*(5), in a study of the red blood cells of 2 patients with hereditary spherocytosis, found an increase in the relative concentration of lysophosphatidyl ethanolamine and a decrease in the relative concentration

of phosphatidyl ethanolamine and postulated that the primary defect in this disorder was a partial block in an enzyme system for conversion of the former to the latter. In the present study, however, the distribution of the phospholipid fractions of the red blood cells of 2 of the patients with hereditary spherocytosis was normal and in the third patient showed some alteration, which may have been due to partial "breakdown" of the unstable "cephalin" fraction during the analytical procedure, a phenomenon that has been repeatedly observed in this laboratory.

A possible explanation for the discrepancy in findings is that part of the ethanolamine-containing phospholipid measured by Allison *et al.*(4) and Kates *et al.*(5) hydrolyzed during the analytical procedure either before or during the chromatography step. These investigators, in fact, used the quantitative paper chromatography method of Marinetti *et al.*(11) at room temperature, a procedure which has been shown to result in hydrolysis of ethanolamine-containing plasmalogen to lysophosphatidyl ethanolamine(11). This hydrolysis probably results from the exposure of the acid-labile α',β'-unsaturated ether bond of plasmalogens to the acidic chromatographic solvent system and can be diminished by performing the chromatography at lower temperatures(11). The increase in concentration of lysophosphatidyl ethanolamine reported by these authors, therefore, could have resulted from the hydrolysis of ethanolamine-containing plasmalogen and the decrease in concentration of phosphatidyl ethanolamine could have actually represented a decrease in concentration of ethanolamine-containing plasmalogen, since in the method of Marinetti *et al.*(11) the ethanolamine-containing plasmalogen and phosphatidyl ethanolamine have the same R_f and are measured together. That these investigators might have been dealing with an analytical artifact is further suggested by their observation that the increase in the spot containing lysophosphatidyl ethanolamine "was exactly balanced" by the decrease in phosphatidyl ethanolamine(4, 5). Ethanolamine-containing plasmalogen has been reported to make up 14% of the phospholipids of normal human red blood cells(12), which is more than enough to account for the amount of lysophosphatidyl ethanolamine found by these investigators.

The 3 patients with hereditary spherocytosis in the present study and one of the 2 patients studied by the above investigators had had splenectomies, but this procedure would not be expected to alter any basic red blood cell defect. If the findings of Allison *et al.*(4) and Kates *et al.*(5) are substantiated, then the possibility arises that more than one type of hereditary spherocytosis exists.

In patients with pernicious anemia, Williams *et al.*(2) observed a slight decrease in red blood cell total and individual phospholipid concentrations; but during treatment to complete remission, an abnormal distribution occurred characterized by a rise in "cephalin" and sphingomyelin and a decrease in lecithin concentrations. In the present study, although the total red blood cell lipid phosphorus concentration may have been slightly reduced in the patients with untreated pernicious anemia, no difference was evident in distribution of the individual phospholipid fractions between the patients with untreated pernicious anemia and those with pernicious anemia in complete remission, both patterns having been normal.

The elevated red blood cell total lipid phosphorus concentration in the patients with intermediate thalassemia and sickle cell anemia is unexplained. The apparently decreased packing ability of sickle cells(13) would tend to lower this value. The young red blood cell population in these patients, however, could at least partially account for this finding, as Prankerd(14) has reported that younger red blood cells have a higher concentration of lipid phosphorus than older.

The normal distribution of the individual phospholipid fractions in the abnormal red blood cells studied indicates that an abnormality in a major phospholipid did not constitute a basic defect in these cells. The basic defect in several of these disorders, nevertheless, may still be in the red blood cell membrane, where important non-lipid components reside.

Summary. The red blood cell phospho-

lipids were analyzed in patients with hereditary spherocytosis, pernicious anemia, sprue, intermediate thalassemia, sickle cell anemia, and polycythemia vera using chromatography on silicic acid columns. The total lipid phosphorus concentration was increased in the patients with intermediate thalassemia and sickle cell anemia and may have been slightly decreased in the patients with pernicious anemia and sprue.

No consistent difference from normal in distribution of the individual phospholipid fractions was found. Variations in the absolute concentrations of the individual phospholipid fractions reflected differences in the total red blood cell lipid phosphorus concentrations.

ADDENDUM. The red blood cells of a 56-year-old lady with paroxysmal nocturnal hemoglobinuria were analyzed and found to contain 4.14 mmoles/l lipid phosphorus with a phospholipid distribution as follows: "cephalin"—44.4%; lecithin—34.1%; sphingomyelin—20.0%; and lysolecithin 1.5%.

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Complement-fixing Antibody Responses to ECHO Virus Types 12 and 19 of Patients with Enterovirus Infections.* (27204)

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Instances of heterotypic complement-fixing (CF) antibody responses in individuals with various enterovirus infections have been cited by several investigators(1-6), some of whom utilized heat-inactivated antigens(1, 2,5), while others employed uninactivated antigens(3,4,6). In our recent study(7) of the antibody responses of individuals with ECHO virus infections, it was noted that a high proportion of the patients studied developed heterotypic CF antibodies to ECHO virus types 12 and 19 (uninactivated antigens); heterotypic rises in CF antibody to the 6 other ECHO virus types used as antigens were infrequently seen. An extension of that work to determine the frequency with which CF antibody to ECHO virus types 12 and/or 19 appears in individuals with enterovirus infections caused by a variety of immunotypes is reported here.

Materials and methods. Human sera examined. Sera examined for presence of CF antibodies to ECHO virus types 12 and 19 were from patients with enterovirus infections confirmed by isolation of the etiologic agent and, in most instances, by demonstration of a diagnostically significant increase

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