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Research Article

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RED-CELL AND PLASMA LIPIDS IN ACANTHOCYTOSIS *

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The rare syndrome of acanthocytosis was first described in 1950 by Bassen and Kornzweig (1) and two years later by Singer, Fisher, and Perlstein (2). The principal manifestations reported were steatorrhea, "atypical" retinitis pigmentosa, progressive neurological deficits, and "thorny" red cells. The most complete case reports to date are those of Meir, Schwartz, and Boshes (3) and Rey (4), who well review the clinical aspects. The erythrocytes of affected individuals have characteristic, spikelike excrescences, a normal or decreased life-span, probably normal osmotic fragility, normal acid fragility, increased susceptibility to mechanical trauma, and an increased rate of destruction in lysolecithin hemolysis tests (2–5).

Recently, it has been demonstrated electrophoretically, immunologically, and by ultracentrifugation that beta or low-density lipoproteins are absent or very low in acanthocytosis (4, 6, 7), thus explaining the low levels of plasma total lipid, cholesterol, and phospholipid noted earlier (3, 8, 9). Striking vacuolization of the intestinal absorptive cell has also been described, and histochemical techniques have shown lipid in these vacuoles (4, 6, 7).

On the basis of these findings, it has been suggested that acanthocytosis is primarily a disease of lipid or lipoprotein metabolism and that the principal clinical manifestations (neurological, hematological, and gastrointestinal) are all secondary to the absence of low-density lipoprotein (6, 7). This is still conjectural, however, and the biochemical defect responsible for the disease has not been elucidated.

The clinical and chemical abnormalities of acanthocytosis suggested that it might be an ideal syndrome in which to seek abnormalities in red-cell lipids. Values previously reported for total red-cell lipids in one case (3) and for red-cell cholesterol and total phospholipid (6) in another were probably normal (normal values were not given), but the possibilities of qualitative changes were not investigated. In the present study, red-cell and plasma lipids from three patients with the disorder were examined, and abnormalities in both the distribution of phospholipids and in the content of esterified linoleic acid have been found. Part of these studies have been previously reported in preliminary form (10), and subsequently Phillips (11) has reported partially confirmatory studies on red-cell and plasma lipids in acanthocytosis.

METHODS

Patient 1 was a preadolescent male,[1] not previously reported, with well-documented acanthocytosis (see Appendix). Patient 2[2] was reported in abstract form by Mabry, DiGeorge, and Auerbach (7, 12). Patient 3[3] was reported by Mier and associates (3). Table I lists the principal manifestations of acanthocytosis found in each of these patients.

Blood was drawn into acid citrate dextrose,[4] refrigerated at 4° C, and transported as directly as possible to

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[1] Under the care of Dr. Charles U. Lowe, Buffalo, N. Y.

[2] Original material kindly furnished by Dr. Arthur McElfresh of St. Christopher's Hospital, Philadelphia, Pa.

[3] Blood obtained through the kindness of Dr. M. Mier, Chicago, Ill.

[4] Formula A. National Institutes of Health, Bethesda, Md.

TABLE I

Principal manifestations of acanthocytosis

Patient	Year of birth	Steatorrhea	Disease of central nervous system	Retinitis pigmentosa	Acanthocytes	Fat absorption	Serum lipids	Lipoproteins
1	1954	Almost constant at age 1 to 4, intermittent since then	Absent deep tendon reflexes throughout; absent vibratory sense and poor position sense in lower extremities	Not at this time	Present now and known since age 2½	Absorbs only 80% of 19-g fat diet Pancreatic enzymes normal Jejunal biopsy shows vaculoes	Very low total lipid, hypocholesterolemia, low phospholipid, altered lecithin: sphingomyelin ratio	Low-density absent by ultracentrifugation and immunoelectrophoresis
2 (7, 12)*	1947	Present now, ? duration	Bedridden at age 13; dysarthria	Not at this time	Present now and known since age 6	Increased fecal excretion of I^{131}-labeled triolein and oleic acid; increase in total fecal fat Jejunal biopsy shows vacuoles	Same changes as above	Low-density absent by electrophoresis
3 (3)*	1943	Present from infancy until adolescence, now improved	Strabismus at 14, unsteady gait at 17; progressive disability subsequently	First noted at 17	"Anemia" as infant; acanthocytes at 17, not definitely excluded before then	Definitely low absorption of I^{131}-triolein and vitamin A	Same changes as above	Low-density virtually absent by ultracentrifugation

* Numbers in parentheses are references to previous reports of the same patient.

one of our laboratories. The longest interval between the time of blood collection and red-cell extraction (or in one instance, freezing) was 15 hours. We have demonstrated that there is no significant change in red-cell total lipids, lipid phosphorus, phospholipid distribution, or fatty acid composition during storage at 4° C in acid citrate dextrose for this length of time. Plasma and red cells were separated by centrifugation at 4° C, and the cells washed three times and resuspended in cold 0.9% NaCl to a hematocrit of approximately 50. After mixing, samples were pipetted for red-cell counts, lipid extractions, and hematocrit. The extraction and subsequent analysis of total lipid, cholesterol, and phospholipids in the Rochester laboratory have been described (13). In the Seattle laboratory, red cells from Patient 1 were similarly extracted. In Patient 3, however, after the reextraction with chloroform, nonlipid impurities were removed by dissolving the lipid in chloroform:methanol:0.1 M KCl 8:4:3 (final relative volumes). After shaking, cooling to 4° C, and rewarming to 25° C, clean separation occurred, and the chloroform layer was recovered. All extractions were performed in duplicate.

In Seattle, lipids were fractioned on silicic acid columns (2 g silicic acid and 1 g of Johns-Manville Hi-Flow Super Cell per 1 mg lipid phosphorus). Neutral lipids were removed with chloroform. The first phospholipid peak, eluted with chloroform: methanol (C: M), 9: 1 (vol: vol), comprised 1 to 2% of the total lipid phosphorus. Although not fully characterized, it has the mobility on paper chromatograms of phosphatidic acid or "polyglycerol phosphatide" (14). The next peak, eluted with C: M, 5: 1, or 6: 1, contained primarily phosphatidyl ethanolamine with some phosphatidyl serine.[5] The third peak, eluted with the first four to five column volumes of C: M or ethyl acetate: methanol, 5: 4, was predominantly phosphatidyl serine by paper chromatography, but also contained phosphatidyl inositol and other compounds as yet unidentified. Continuing the same solvent, a large fraction was then eluted, the first two-thirds of it chromatographically pure lecithin. Subsequently, a mixture of lecithin and sphingomyelin appeared, the solvents were changed to C: M, 1: 9, and the final peak, approximately 90% sphingomyelin and 10% lecithin, emerged. Each peak containing more than one phospholipid was rechromatographed on paper by the method of Reed, Swisher, Marinetti, and Eden (13), and lipid phosphorus was eluted quantitatively. These data, in addition to molar nitrogen:phosphorus and molar fatty acid ester:phosphorus ratios, were used to arrive at the final phospholipid partition.

Phospholipid fatty acids were methylated by dissolving the lipid in 0.5 ml benzene, adding 2 ml of absolute methanol and 0.04 ml of concentrated sulfuric acid, and then refluxing 4 hours at 60° to 70° C. The methyl esters were extracted with petroleum ether, washed with water four times, dried over anhydrous sodium sulfate, and then dissolved in redistilled hexane. Gas-liquid chromatography of the methyl esters was performed on either a Barber-Colman model 15 or a Pye-Argon gas chromatograph with ethylene glycol succinate as stationary phase at column temperatures of 165° to 172° C and gas inlet pressures of 12 to 20 pounds per square inch. Methyl esters of myristic, palmitic, stearic, oleic, and linoleic acids pure by thin-layer and gas-liquid chromatography were used to standardize the instrument, and the molar percentages for these five compounds are correct to ± 2%. In calculating methyl arachidonate and longer-chain methyl esters for which standards were not available, the molar percentage of each ester was assumed to be proportional to its peak area when the Pye-Argon equipment was used, and proportional to peak area divided by molecular weight with the Barber-Colman instrument (flame detector. Red-cell ghosts were prepared in 20-milliosmolar phosphate buffer at pH 7.4 by the method of Dodge, Mitchell, and Hanahan (16), and red-cell counts were done on a Coulter electronic counter. Fatty acid esters were quantitated by dissolving the lipid in chloroform and measuring infrared absorption at 5.75 μ in 1-mm NaCl cells with a Perkin-Elmer model 21 infrared spectrophotometer. Calculations were made from the optical density of appropriate standards. This method has been shown to be linear between 2 and 22 μEq fatty acid ester per ml for triolein, dimyristoyl lecithin, and cholesterol palmitate. Plasmalogens (17), ultracentrifugation of plasma lipoproteins (18), lipid phosphorus, nitrogen, total solids, and cholesterol (15) were done by established procedures.

Certain of the total lipid analyses and phospholipid partitions were performed in both laboratories on different samples of the same blood. When such data have been averaged, this is indicated in the tables.

RESULTS

Red-cell and plasma lipid studies in patients with acanthocytosis

Total lipids and phospholipid distribution. Previous publications have characterized and quantitated the major lipid classes in human red cells (13, 15, 19–21). More than two-thirds is phospholipid, 25% neutral lipid (primarily cholesterol with some glycerides and cholesterol esters), and 8 to 10% is not yet fully characterized, but is high in carbohydrate. When normal red-cell phospholipids are partitioned, lecithin is found to be the most abundant of the red-cell phosphatides, sphingomyelin and ethanolamine phosphoglycerides are present in almost equal amounts, serine phosphoglycerides comprise 12 to 18% of the total phos-

[5] While the presence of free serine and ethanolamine in hydrolyzed material of this fraction has been confirmed in normal cells (15), this analysis was not carried out in the acanthocytes. On both silicic acid-impregnated paper and thin-layer plates, however, the intact lipid gave only two Ninhydrin-positive spots with the mobility of phosphatidyl serine and phosphatidyl ethanolamine.

TABLE II

*Red-cell lipids in acanthocytosis**

	Normal		Acanthocytosis			Family of Patient 1		
	Mean	1 SD	Patient 1†	Patient 2	Patient 3	Father	Mother	Brother
Total lipid per cell, $mg \times 10^{-10}$	4.83 [13]	.25	5.37	4.60	4.98	4.90	5.08	5.12
Lipid phosphorus per cell, $mg \times 10^{-11}$	1.16 [13]	.06	1.15	.97	1.20	1.26	1.25	1.20
Total cholesterol per cell, $mg \times 10^{-10}$	1.18 [9]	.06	1.28	1.23	1.09	1.23	1.30	1.11
Plasmalogen per cell, $\mu M \times 10^{-11}$	5.01 [9]	.51	3.40		3.14	4.75	5.67	5.11
Lipid phosphorus distribution, *% of total recovered*	[11]							
Lecithin	29.5	1.5	19.9	18.4	15.0	28.8	32.4	30.5
Sphingomyelin	23.8	1.7	31.4	36.2	28.5	25.3	23.2	22.5
Ethanolamine phosphoglycerides	25.7	2.6	22.9	19.5	24.0	23.6	21.0	25.1
Serine phosphoglycerides	15.0	1.6	15.0	15.9	17.5	14.4	15.9	15.9
Other	5.9	1.7	9.0	9.3	15.0	9.0	7.6	6.4
Total recovery	95	3	93	101	96	105	108	107

* Normal values given are from the Seattle laboratory. Numbers in brackets after normal mean values represent the number of individuals upon whom the values are based. Normal values from the Rochester laboratory have been published and are comparable (13).
† Values for Patient 1 are averaged from assays done in both laboratories, those for Patient 2 were all done in Rochester, and those for Patient 3 and the family study, all in Seattle.

pholipid, and the remainder is phosphatidyl inositol, lysolecithin, and other minor components.

In Table II, the values obtained for total lipid, total lipid phosphorus, total cholesterol, and plasmalogens per red cell in three patients with acanthocytosis are compared with the corresponding normal values. In Patient 2, lipid phosphorus and total solids were low. This patient also has sickle-cell trait, but it is not known whether this could account for these differences. The values for red-cell plasmalogens were significantly decreased in the two cases assayed.

Quantitation of each phospholipid class (Table II) revealed a consistent and significant decrease

TABLE III

*Plasma lipids and lipoproteins in acanthocytosis**

	Plasma lipids			Phospholipid partition†			Ultracentrifugal fraction: Density <1.063		Ultracentrifugal fraction: Density >1.063	
Subject	Total	Lipid phosphorus	Cholesterol	Lecithin	Sphingomyelin	Other	Lipid phosphorus	Cholesterol	Lipid phosphorus	Cholesterol
	mg/100 ml			*% of total phospholipid*			*mg/100 ml*		*mg/100 ml*	
Patient										
1		1.8	34	55	29	16				
		2.0	37				.06	2.2	1.97	27
2	180	4.2	59	49	47	4.0				
3	96	1.3	26	42.5	35.5	22	.004	.8		
Family of Patient 1										
Father (age 35)‡		7.9	193	65.0	21.6	13.4				
		9.3	206				3.5	127	5.6	68
Normal 1‡		9.3	200				3.3	111	5.3	63
Mother (age 35)‡		10.0	200	70.2	19.4	11.4	2.9	101	6.2	77
Normal 2‡		10.3	187				3.0	98	6.5	71
Brother		6.9	181	64.9	21.5	13.6	2.3	123		
Normal, Havel (17)										
Men		8.7	172				4.0	125	4.6	46
Women		9.2	178				3.7	116	5.4	57

* All analyses were performed in Seattle except those on Patient 2 (Rochester) and the phospholipid partition in Patient 3 (average of Rochester and Seattle values).
† Percentage of total lipid phosphorus recovered from the chromatograms.
‡ The ultracentrifugal analyses on the father and mother were performed simultaneously with normal subjects 1 and 2, respectively.

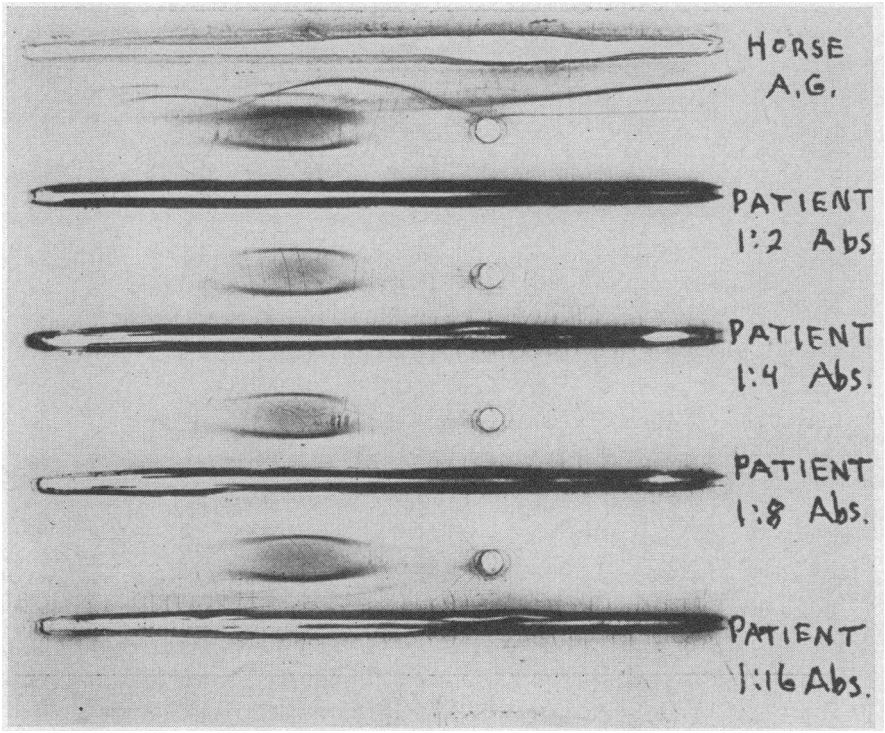

FIG. 1. IMMUNOELECTROPHORESIS OF SERUM OF PATIENT 1. Top trough contains horse antihuman globulin serum; subsequent troughs contain the same serum absorbed with serial dilutions of patient's serum. Normal human serum was placed in each well. The plate was stained with lipid crimson and trifalgic acid. The more concentrated patient's serum absorbed all the antibodies except those to beta lipoprotein, as the prominent lipid-staining band in the low-density region shows.

in the relative and absolute amount of lecithin present, and a corresponding increase in sphingomyelin. Similar changes were found in the distribution of plasma phospholipids (Table III). In normal plasma, lecithin is two to four times as plentiful as sphingomyelin (22, 23), but in acanthocytosis, both plasma lecithin and sphingomyelin were very scarce, and the lecithin: sphingomyelin ratio averaged only 5:4.

In Patients 1 and 3, serum lipoprotein distribution was studied by ultracentrifugation (Table III). The amount of lipid phosphorus and cholesterol detected in the upper layer at a density of 1.063 after centrifugation was minimal and could have resulted from mixing in the tube during the slicing procedure. As noted by others, the total levels of serum lipid phosphorus and cholesterol in these patients are compatible with decreased levels of high-density lipoprotein, in addition to absence of low-density lipoprotein (4, 6).

Paper and cellulose-acetate electrophoresis were performed on the serum of Patient 1. No lipid-staining material was seen in the low-density lipoprotein area. Figure 1 demonstrates even more conclusively the absence of low-density lipoprotein. Immunoelectrophoresis of normal serum was performed against a horse antiglobulin serum absorbed in troughs 2, 3, 4, and 5 with decreasing concentrations of the patient's serum. In contrast to the unabsorbed antiserum, which produced numerous protein bands in addition to the beta-lipoprotein band, the next two show only the beta-lipoprotein band, indicating that the patient's serum was unable to absorb the antibodies to the low-density lipoprotein. As the patient's serum was diluted more, the other reaction bands were again seen.

Fatty acid composition. Red-cell total phospholipid was transesterified, and the resulting methyl esters were analyzed (Table IV). The striking change was in the percentage of linoleic acid, which in the acanthocytes was not in excess of

TABLE IV

Principal fatty acids esterified to total red-cell phospholipid

Fatty acid	Normal range (8 persons)	Acanthocytosis			Family of Patient 1		
		Patient 1	Patient 2	Patient 3	Father	Mother	Brother
		moles/100 moles total fatty acid			*moles/100 moles total fatty acid*		
Palmitic (16:0)	23.8–26.0	27.5	27.6	30.1	23.6	26.3	22.0
Stearic (18:0)	17.0–21.0	15.0	22.3	19.7	19.5	18.7	19.7
Oleic (18:1)	15.0–18.1	21.9	22.7	17.6	17.5	14.6	16.3
Linoleic (18:2)	9.7–13.5	1.8	1.7	2.8	11.3	11.5	11.1
Arachidonic (20:4)	12.2–15.9	5.4	10.1	9.3	16.1	10.6	15.4

3%, whereas normally it comprises 9.7 to 13.5% of the total.[6] Since the total amount of phospholipid per cell was normal or decreased in all three patients, this deficiency was absolute as well as relative.

In two patients, the fatty acids of the individual phospholipids were analyzed, and in each major phospholipid class, the amount of linoleic acid was approximately one-fourth that normally seen. This was most clearly demonstrated in the lecithin fraction, where linoleic acid, normally 18 to 25% of the fatty acid present, was decreased to 5% or less in both patients (Table V). In the same two patients, plasma cholesterol esters were isolated and the fatty acids examined. This lipid class, normally rich in linoleic acid (50% or more) (15), contained only 9.1 and 9.8% linoleic acid in Patients 1 and 3, respectively. Plasma lecithin and sphingomyelin were correspondingly low in linoleic acid.

TABLE V

*Fatty acids esterified to red-cell lecithin**

Fatty acids	Normal range (12 persons)	Acanthocyte	
		Patient 1	Patient 3
	moles/100 moles total fatty acid		
Palmitic (16:0)	30.7–40	46.0	47.0
Stearic (18:0)	11.3–16.2	9.0	9.4
Oleic (18:1)	18.1–24.7	34.0	28.5
Linoleic (18:2)	17.8–24.7	5.0	4.5
Arachidonic (20:4)	3.0–9.5	5.0	8.0
Other		1.0	2.6

* In Patient 1, the lecithin isolated from the column was pure by paper chromatography and had a fatty acid ester: phosphorus molar ratio of 1.90. In Patient 3, the "lecithin" contained 17% phosphatidyl serine (phosphorus elution from silicic acid paper chromatography), had a nitrogen: phosphorus molar ratio of 1.01, and a fatty acid ester: phosphorus molar ratio of 1.90.

[6] In our abstract (10), one value higher than 3% was reported. This assay was carried out on a lipid sample refrigerated 6 months before the methyl esters were made. When a freshly extracted phospholipid sample from the same patient was transesterified and measured, the value for linoleic acid was 1.8%.

Lipid composition of acanthocyte ghosts ("membrane preparations"). Recently, Dodge and coworkers (16) and Weed, Reed, and Berg (24) have prepared hemoglobin-free, red-cell ghosts containing all of the lipid phosphorus and cholesterol that is extracted from intact red cells. Phase micrographs of such preparations demonstrate that most of the ghosts are intact discoid bodies without the refractile properties imparted to the normal red cell by hemoglobin (16).

In the present study, it seemed desirable to ascertain the lipid composition of such membrane preparations. Therefore, ghosts containing less than 1% hemoglobin by weight were prepared from the red cells of Patients 1 and 3. Phase micrographs revealed that these acanthocyte "membranes" were indistinguishable from normal ghosts. Total phospholipid, total cholesterol, and the abnormal distribution of lecithin and sphingomyelin were the same as in the intact acanthocyte. Fatty acid analyses of ghost lipid also showed decreases in linoleic acid identical to those in lipid extracted from intact acanthocytes.

Specificity of the lipid abnormalities in acanthocytosis

Findings in patients with other types of steatorrhea, or malnutrition, or both. Because of the gastrointestinal manifestations of this disease and the abnormal histological picture of the gastrointestinal mucosa, it has been suggested that steatorrhea per se or changes in dietary habits because of steatorrhea might be the cause of the low red-cell linoleic acid. Although the exact dietary composition of these patients at the time their blood was analyzed is not known, Patient 1 was

TABLE VI

Red-cell lipids in patients with malabsorption, or malnutrition, or both

Patient	Age	Diagnosis	Lipid phosphorus	Cholesterol	Phospholipid distribution*					Linoleic-acid esterified to phospholipid
					PE	PS	Lec.	SP	Other	
	years		*mg × 10⁻¹⁰/cell*		*% of total phospholipid*					*moles/100 moles total fatty acid*
1	10	Cystic fibrosis	.123	1.25			28.3	23.8		9.8
2	70	Celiac sprue	.156	2.17	35.7		34.3	25.1	5.1	7.7
3	40	Steatorrhea, secondary to small bowel resection	.123		30.8	21.2	25.7	15.3	7.2	5.8
4	60	Folic acid deficiency, malnutrition	.110	1.40	21.9	18.4	32.4	15.1	12.4	5.0
5	50	Chronic relapsing pancreatitis with steatorrhea and malnutrition	.155	1.73	22.1	16.4	28.1	23.1	10.3	6.0
6	12	Steatorrhea and growth failure, unknown etiology	.123	1.22	23.2	16.5	31.3	25.2	4.0	

* Percentage of total lipid phosphorus recovered; PE = phosphatidyl ethanolamine, PS = phosphatidyl serine, Lec. = lecithin, and SP = sphigomyelin.

only eating 19 g of fat daily 1 year after these studies. Similarly, Patient 3 is described as avoiding fat (3). To evaluate the possibility that the red-cell and plasma lipid abnormalities might be nonspecific, red-cell lipids from other patients with malnutrition, or steatorrhea of other etiology, or both, were analyzed (Table VI). The percentage of red-cell linoleic acid was reduced in several instances, but in no case was this change of the magnitude seen in the patients with acanthocytosis. In contrast to acanthocytosis, the lecithin : sphingomyelin ratio was not reversed in the red cells of any of these patients. In fact, in two of the five studied (Patients 2 and 4), there was actually an increase in the amount of lecithin normally found, and in one of these, a corresponding decrease in the amount of sphingomyelin.

Lipid analysis of red-cell populations high in reticulocytes. One of the difficulties inherent in studies of red-cell metabolism is the necessity of dealing with cells heterogeneous with respect to age. When average cellular age is shortened by accelerated *in vivo* destruction, as it appears to be in some cases of acanthocytosis (3, 4), it could be argued that any changes found in red-cell lipids were merely a function of decreased mean cellular age.

In five instances, the erythrocyte phospholipid distribution of patients with reticulocytosis was determined by paper chromatography (Table VII). There was no significant difference between this group and normal mature cells in the percentage distribution of the major erythrocyte phosphatides. The fatty acid distribution of the red-cell phosphatides was determined in two patients of this group, and it indicates that young cells are lower in linoleic acid than a cell population of normal age distribution.

Family studies

Since previous investigations have suggested that acanthocytosis is an inherited disease (1, 2, 4, 6), the father, mother, and brother of Patient 1 in the present investigation were studied. Red-cell morphology, total lipid, phospholipid distribution, and plasmalogen levels were normal in all three family members (Table II), as was the fatty acid composition of total red-cell phospholipid (Table IV).

Plasma lipid and lipoprotein values for the family members are shown in Table III. The parents had normal total lipid values, total cholesterol, lipid phosphorus, and phospholipid distribution. Analysis of serum fractions separated by ultra-

centrifugation gave results comparable to normal sera examined simultaneously and to published normal data (18, 25). In addition, immuno-, starch gel, and cellulose acetate electrophoreses all demonstrated normally staining bands of low-density lipoprotein in samples from the father and mother. The brother, however, had low cholesterol and slightly low lipid phosphorus on one examination. A normal percentage of this lipid was found in the density layer of less than 1.063, and as with the parents, none of the electrophoretic studies was abnormal. Repeated analyses of the brother's plasma on two subsequent occasions gave values of 6.55 and 6.90 mg per 100 ml for lipid phosphorus, and 170 and 181 mg per 100 ml for cholesterol.

DISCUSSION

The present study establishes that red cells in the hereditary syndrome of acanthocytosis contain increased amounts of sphingomyelin, are deficient in lecithin and plasmalogens, and contain very little esterified linoleic acid. These same deviations in phospholipid composition and linoleic acid content were found in hemoglobin-free ghosts, indicating that the lipid composition of the membrane itself is abnormal.

The significance of these observations for acanthocytosis depends largely on their peculiarity to this syndrome. Linoleic acid, which probably cannot be synthesized by mammals, can be reduced experimentally in animal erythrocytes to levels as low as those found in the acanthocyte. Thus, in rats (26) and monkeys (27–29), decreased percentages (absolute levels were not measured) of red-cell linoleate follow prolonged dietary restriction of linoleic acid. Similarly, our analyses of red cells from other subjects with steatorrhea, or malnutrition, or both, generally showed reduction of red-cell linoleate. In these, however, the decreases were not to the extent observed in acanthocytosis. There is another interesting difference between the deficient animals and the patients with malabsorption (whether due to acanthocytosis or not). In the experimentally deficient animals, there is marked increase of a red-cell fatty acid with the chromatographic retention characteristics of eicosatrienoic acid (27–29). This increase is now considered an integral feature of essential fatty acid deficiency, but it was not seen in either the patients with acanthocytosis or those with malabsorption of other etiology.

In contrast to the low levels of linoleate, the abnormal phospholipid distribution found in acanthocytosis has not, to our knowledge, been described previously in human red cells. Although phospholipid studies were not performed in the animal work discussed above, analyses of red cells from patients with other types of steatorrhea indicate that an increase in red-cell sphingomyelin and a decrease in lecithin are not functions of malabsorption or fat deprivation per se. This evidence suggests that a defect in linoleate absorption or metabolism is not the primary defect in acanthocytosis, but that the abnormalities in phosphatide distribution may be specific and consequently of more fundamental importance.

TABLE VII

Lipid analysis of young red cells

Patient	Diagnosis	Reticulocytes	Phospholipid distribution			Esterified linoleic acid
			Lecithin	Sphingomyelin	Other	
		% of total cells		*% of total*		*moles/100 moles total fatty acid*
1	Myeloproliferative disease with gastrointenstinal bleeding	10	32.2	25.8	42.0	6.0
2	Treated folic acid deficiency	22	31.1	27.0	42.3	5.1
3	Hereditary spherocytosis (pre-splenectomy)	16	32.0	21.0	47.0	
4	Acquired hemolytic anemia (Coombs-positive)	23	29.0	20.0	51.0	
5	Treated pernicious anemia	20	27.0	19.0	54.0	

Erythrocyte plasmalogens, which were also decreased in the acanthocyte, were determined in only two of the other types of malabsorption, and on a per cell basis were normal. Since in normal red cells 8 to 10% of total plasmalogens are in the lecithin fraction (19), part of their decrease in acanthocytes may be related to the deficiency in red-cell lecithin. It can, however, be calculated that the observed decrease is greater than that expected even if all of the lecithin plasmalogens were absent. Further studies are required to elucidate the association between low plasmalogens and the phospholipid abnormality.

Another factor influencing the linoleic acid content of red cells is their average age. Reticulocyte counts, bone marrow examinations, and chromium survival curves were available for two of our patients. They indicated that the acanthocyte probably does not survive normally *in vivo,* and similar findings have been reported by others (3–5). Since the average age of red cells is known to influence their fatty acid composition (30), reticulocyte-rich blood from several persons was examined by the same techniques used to analyze the acanthocytes. In these subjects the reticulocyte count was two to six times higher than in any of the patients with acanthocytosis. Although a decrease in linoleic acid content was observed, it did not reach the low values found in acanthocytosis. Therefore, the linoleic acid level observed in Patients 1 and 3 is probably lower than would be expected on the basis of cellular age alone, but may be consistent with decreased mean cellular age in a patient also on low-fat intake. The reticulocyte studies, however, again support the specificity of the phospholipid abnormality, since the lecithin and sphingomyelin distribution of red-cell populations high in reticulocytes is the same as in the mature cell.

In any cell bathed continuously with plasma, it is reasonable to suppose that the original membrane lipid composition might be susceptible to alteration by the environment. An exchange between plasma and red-cell lipid phosphorus equal to 10% of lipid phosphorus in the cell daily has now been documented (31), and previous workers have shown rapid exchange of plasma and red-cell cholesterol both *in vivo* and *in vitro* (32, 33). Since total plasma lipid in acanthocytosis is unusually low, and plasma cholesterol, lecithin, and sphingomyelin are deficient in absolute amount and altered in distribution, it is conceivable that the replenishment of membrane lipid in this disease could be seriously impaired, with resultant changes in red-cell phospholipids. On the other hand, defective membrane synthesis in the marrow, or the combination of defective synthesis and exchange, might produce the same defects. None of the present data indicate which possibility is most likely. The morphology of marrow cells in this disease, however, has invariably been normal (2–4). Also, DiGeorge, Mabry, and Auerbach have reported normalization of cell morphology after 4 to 6 weeks of Lipomul therapy (12). Farquhar has noted immediate restoration of the acanthocyte to normal red-cell shape *in vitro* after the addition of Lipomul (34), and Switzer has shown that Tween 80 is the component of Lipomul which produces this change (35). These findings are compatible with, although they are not direct evidence for, an important environmental contribution to the abnormalities observed. The phospholipid composition of young red cells and other tissues is now under study in an effort to clarify the relative importance of environment and de novo synthesis in the abnormalities of acanthocyte lipid.

The plasma lipid abnormalities in acanthocytosis deserve further comment. Both this study and the work of Phillips (11) show that the very low levels of plasma cholesterol and total phospholipid are accompanied by an increase in the percentage of sphingomyelin and a corresponding decrease in lecithin—a change qualitatively identical to that in the acanthocyte. Phillips (25), however, in an earlier study and others (36, 37) have shown that in normal blood the lipoproteins of density greater than 1.063 contain relatively *more* lecithin than those of density less than 1.063. Thus, the relative increase in plasma sphingomyelin found in acanthocytosis is opposite to what might have been expected in a condition where the low-density plasma lipoproteins are decreased or absent. Hence, the high-density lipoproteins in acanthocytosis are not only low in absolute amount (see Results), but qualitatively abnormal as well —a finding which suggests that the phospholipid abnormalities may play a more basic role in the

pathogenesis of the disease than previously supposed.

These and other studies (6, 11) have disclosed several chemical abnormalities in the red cells and plasma of afflicted persons that theoretically might provide genetic markers for detecting heterozygous carriers of the disease. Also, if any one of the defects (abnormal distribution of phospholipids, low linoleic acid, decreased low-density lipoproteins, or low red-cell plasmalogens) were present in the heterozygote to the exclusion of the others, this could provide an important clue to the basic biochemical defect in acanthocytosis. While Salt and his colleagues were able to demonstrate half-normal concentrations of beta lipoprotein in the parents of their patient (6), neither we nor Rey (4) has been able to confirm these findings. Likewise, our plasma lipid assays and red-cell analyses have not demonstrated any qualitative or quantitative abnormality that would seem indicative of a heterozygous defect in either of the parents. The discrepancy between Salt's data and our own may indicate that this is an abnormality of variable penetrance, or that more than one type of acanthocytosis exists.

The older view of the red-cell membrane as a bimolecular leaflet of lipid in combination with protein, as originally espoused by Ponder and others (38), has gained new credence from Robinson's concept of the unit membrane evolved through electron microscopic study of cell structure (39). In this synthesis, protein, lipid, and carbohydrate all play a major role in the structure of membranes. Potentially, abnormalities in any of these entities might be responsible for defects of red-cell structure or function. The present studies have demonstrated defects of lipid composition in erythrocytes that are both functionally and morphologically abnormal. Although it remains to be established that this is a cause-and-effect relationship, the prospect is intriguing.

Finally, it is tempting to speculate that the lipid and lipoprotein abnormalities of acanthocyte membranes may reflect changes in lipid composition of other cell membranes and are, consequently, related to the other clinical manifestations, particularly the neurological disease. The morphological abnormality of the red cells in acanthocytosis provided impetus for investigation of their lipid composition. The finding that red-cell lipids are abnormal in one disease with overt and eventually disabling neurological manifestations should provide an incentive to the careful study of the red-cell membrane in other neurological syndromes of obscure etiology.

SUMMARY

The red-cell and plasma lipids from three patients with acanthocytosis have been studied and definite abnormalities found. *1*) In the presence of normal or reduced values for total lipid, lipid phosphorus, and cholesterol, the distribution of red-cell or ghost phospholipids is altered, with an increase in sphingomyelin and a decrease in lecithin. Sphingomyelin was the predominant phospholipid in all three sets of red cells studied. *2*) The plasma phospholipids were reduced in total amount to less than 20% of normal values and also manifested significant percentage increases in sphingomyelin, with correspondingly less lecithin. *3*) The esterified linoleic acid content of red-cell total phospholipid, individual red-cell phospholipids, and various plasma lipid fractions was decreased to 25% or less of the amount normally found. *4*) In patients with steatorrhea of other etiology, red-cell linoleic acid was decreased, but not to the extent noted in acanthocytosis, and this change was never associated with a reversal in the amounts of lecithin and sphingomyelin. *5*) Similarly, analysis of reticulocyte-rich blood revealed lower levels of linoleic acid, but normal distribution of phospholipids. *6*) Studies of red-cell and plasma lipids and plasma lipoproteins in the parents and a sibling of one patient failed to reveal any abnormalities. *7*) These results suggest that the alterations in phospholipid distribution seen in this syndrome may be specific, but that the low levels of linoleic acid could be caused by decreases in available exogenous linoleate, or a younger than normal population of red cells, or both.

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APPENDIX

Report of Patient 1

Growth and development. This white male child was born in 1954, the second son of Jewish parents. A prominent abdomen and rather thin extremities were noted at birth. During the first two months of life, he required hospitalization for vomiting, which subsided after numerous qualitative manipulations in his diet. He grew slowly and was significantly delayed in sitting, standing, and walking. At 3½ years of age, his head circumference was 2.5 SD less than normal, and his length and weight were 1.5 SD less than normal. A skeletal survey at that time reported no retardation of bone age, but subsequently the child has remained short and thin for his age. Now 8 years old, he is in the third percentile for both height and weight.

Gastrointestinal disease. At birth the boy began to have frequent, loose, pasty, foul-smelling bowel movements, projectile emesis, and a protuberant abdomen. During his first hospitalization (at 2 months), he improved on a diet of skim milk and molasses. Because of "malnutrition," however, he was hospitalized at 2½ years of age at Babies' Hospital in New York under the care of Dr. James A. Wolff. During this hospitalization, abnormal red cells were first noted, and the diagnosis of acanthocytosis with "celiac-like syndrome" was made by Dr. Wolff. On a "regular diet," the diarrhea subsided, to return only sporadically. Evaluation at the Buffalo Children's Hospital about a year later (at 3½ years) revealed a flat vitamin A tolerance curve and normal glucose tolerance. At age 6, a small-bowel biopsy (Dr. Charles Lowe) revealed vacuolization of intestinal absorptive cells identical on hematoxylin-eosin-stained sections to that reported by Salt and co-workers shortly thereafter (6). A superficial dietary history at the time of initial red-cell lipid studies suggested that he was ingesting "normal" amounts of fat. Subsequently, more accurate calculations indicate that he has been taking only 10 to 15% of calories as fat. He continues to have occasional episodes of watery diarrhea lasting 1 to 2 days, occurring about once a month. Upper gastrointestinal and small-bowel X rays in 1962 revealed no specific abnormalities.

Neurological disease. When he was hospitalized at 2½ years, no "neurological nor ophthalmological abnormality" was found. One year later at Buffalo Children's Hospital, absent deep tendon reflexes in the lower extremities were noted, with minimal reflexes, if any, in the upper extremities. Definite ataxia was not observed. Spinal fluid and electroencephalogram were normal. Subsequently, according to the parents, muscular coordination has been poor. In 1961, ptosis and strabismus of the left eye were noted, and they have persisted. In retrospect, the mother believes he may have had a "squint" at age 2 that subsequently disappeared. Since 1960, mild fecal stress incontinence and an inordinate ability to retain urine have been noted. Neurological examination in June, 1962, revealed an ataxic gait, decreased muscle tone in all extremities, complete absence of deep tendon reflexes, and a questionable left extensor plantar response. Vibratory sensation was absent at and below the iliac crest and decreased in the elbows and wrists. Position sense and deep pain perception was decreased bilaterally, and the heel-to-knee maneuver was abnormal bilaterally. Sensations of pin prick and light touch were intact throughout, and there were no trophic changes of the skin. An alternating exotropia was present without inequality in pupillary size or abnormality of pupillary response. Vision was equal in both eyes, and no field defect could be demonstrated. Retinoscopic examination was normal.

Hematological disease. Since the abnormal red cells were first identified at age 2½, they have been seen repeatedly. Peripheral white cells, platelets, and on two occasions, all cellular elements of the marrow have been morphologically normal. In June, 1957, hematocrit and reticulocyte count were normal for the patient's age, but in April, 1958, reticulocytes were 2.2% despite normal hematocrit. Chromium $t_{\frac{1}{2}}$ at that time was 22 days (normal, 26 to 27 days).[7] The red-cell and plasma lipids were first analyzed in May, 1960, with the results reported in this publication. In July and August, 1962, hematocrits were 34 to 35%. Reticulocyte counts were 4 to 5%, and the chromium $t_{\frac{1}{2}}$ was again slightly shortened.

ADDENDUM

Farquhar and Ahrens (40) have now reported a level of 3% linoleic acid in the phospholipids of another patient with acanthocytosis. Watson (41) has shown that red-cell lipids of essential fatty acid-deficient rats contain very little linoleic acid, but are evidently normal in phospholipid distribution.

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[7] The results of these studies were kindly furnished by Dr. Clare Shumway, Buffalo, N. Y.

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