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PHOSPHOLIPID COMPOSITION OF DYSTROPHIC CHICKEN ERYTHROCYTE PLASMALEMMAE

I. ISOLATION OF A UNIQUE LIPID IN DYSTROPHIC ERYTHROCYTE MEMBRANES

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Several structural and functional properties are characterized in nucleated erythrocyte plasmalemmae of age- and sex-matched dystrophic (line 413) and normal (line 412) chickens obtained from the University of California at Davis. Plasmalemma purity is assessed through marker enzymes. Significant differences are observed in the phospholipid content between dystrophic and normal chickens. The dystrophic chicken erythrocyte plasmalemma has an increased concentration of phosphatidylserine and a decreased concentration of phosphatidylethanolamine compared with control birds. Also, a measurable and distinct polar lipid, observed only on thin-layer chromatography (TLC) plates spotted with dystrophic preparations, is visualized adjacent to phosphatidylethanolamine. These abnormalities in the dystrophic chicken erythrocyte may signal a general defect in membrane structure for chicken dystrophy.

Introduction

Anomalies in membrane fluidity, membrane structure, and membrane embedded proteins have been characterized in the myopathic chicken [1]. These membrane anomalies are observed in tissue other than muscle, and are the rationale for these chicken erythrocyte membrane studies. Specifically, utilizing electron spin resonance spectroscopy, Butterfield and Leung [2] observe an increased rigidity of erythrocytes in four week ex ovo dystrophic chickens. Sha'afi et al. [3] note an increased microviscosity in dystrophic chicken erythrocyte membranes using fluorescence polarization spectroscopy. Rodan et al. [4] note abnormalities associated with two membrane related proteins (i.e., a decreased activity of adenylate cyclase and an increased activity ($Na^+ + K^+$)-ATPase) in erythrocyte, liver and skeletal muscle preparations from dystrophic chickens. Also, functional studies performed by Kester et al. [5] note anomalies in dystrophic chicken erythrocyte osmotic fragility, half-life and ^{24}Na passive influx compared to age-matched controls.

Although muscle degeneration is the predominant symptom in dystrophy, the erythrocyte, for a number of reasons has been selected by investigators to study membrane pathology [6]. Erythrocytes may be reflective of a muscle membrane defect in dystrophic chickens [1]. The muscle's primary lesion may be masked by infiltration of fat or connective tissue or may affect one fiber type over another [6]. Moreover, separation of sarcolemmae from muscle contractile elements, T-system contaminants, sarcoplasmic reticulum, basal lamina or collagen fibers is difficult [7]. The erythrocyte, however, is a readily accessible cell. An enriched plasmalemma is readily obtained after

osmotic lysis, homogenization or nitrogen cavitation [6]. Since the animals do not have to be biopsied or sacrificed, the same animal can be studied over a period of time. Furthermore, the avian erythrocyte is a nucleated cell containing all the enzymatic machinery for membrane regulation and repair [8]. Moreover, the avian erythrocyte is a dynamic model for membrane assembly and biosynthesis compared with the presumably slow maturation process observed in muscle tissue.

The phospholipid profile of erythrocytes from patients diagnosed with Duchenne dystrophy has been analyzed and contradictory data have been reported. In this studies that did not use antioxidants for erythrocyte preparation an elevated content of sphingomyelin [9,10] and a decreased concentration of phosphatidylserine and phosphatidylethanolamine were noted [10]. However, other studies that utilize anti-oxidants show no significant differences in phospholipid concentrations between normal and dystrophic erythrocytes [11–13]. Oxidation of polyunsaturated fatty acids leads to the apparent loss of their phospholipids in lipid determination [14]. The high values for sphingomyelin in the studies of Kunze et al. [9] may be explained by the fact that the fatty acids of sphingomyelin are highly saturated and would not be subject to significant auto-oxidation. Therefore, in this study, utilizing anti-oxidants, we note that there are significant differences in the phospholipid composition of erythrocyte plasmalemmae between normal and dystrophic chickens. The major classes of phospholipids and their fatty acid profiles are analyzed.

Materials and Methods

Source and care of chickens (Gallus domesticus). One-day-old dystrophic line 413 (early onset, genetically homozygous) and normal line 412 chickens were obtained from the Department of Avian Sciences at the University of California, Davis. All chickens were males. They were maintained in our university facilities at an ambient temperature of 25.5 °C under a 12/12 hour light cycle. the chickens were provided with food and water ad libitum.

Flip testing of chickens. The dystrophic chicken line chosen for this study displayed symptoms which had a reproducible onset and a rate of progression. A periodic flip test offered a method of testing afflicted chickens for functional disability. After 30 days ex ovo, dystrophic chickens begin to lose their ability to right [15]. Normal and dystrophic chickens were placed in a supine position. The number of successful attempts to regain an upright posture scored in five consecutive attempts was designated as the flip number. The investigator used two hands to steady each chicken before the actual flip. Handling was kept to a minimum during the testing procedure.

Preparation of erythrocyte plasmalemmae [16]. Blood samples for erythrocyte plasma membrane preparation were taken from the jugular vein of dystrophic and non-dystrophic chickens, with heparinized syringes. The blood samples were centrifuged at $480 \times g$ at 4 °C for 5 min. The plasma was decanted and frozen at −70 °C for subsequent analysis. The cells were washed four times in cold 150 mM (isotonic) NaCl. The while blood cell layer was removed. The washed pellet was hemolysed in 5 ml of cold hypotonic buffer containing 0.01 M Tris-HCl (pH 7.5), 0.005 M KCl and 0.001 M $MgSO_4$ and centrifuged at 3000 $\times g$ for 10 min at 4 °C. The supernatant fluid was decanted and the hemolysis was continued by adding an amount of hypotonic buffer equal to the volume of decanted supernatant to the pellet. After four such treatments, the sedimented pellet was homogenized in a Sorval omni mixer. This homogenate was diluted in a volume of hypotonic buffer equal to the volume of the homogenate and centrifuged at $4000 \times g$ for 15 min at 4 °C. The supernatant was removed and the pellet was diluted with a volume of hypotonic buffer equal to the volume of the decanted supernatant. The suspension was recentrifuged at $4000 \times g$ for 15 min at 4 °C. The white middle membranous layer was removed gently. This membrane layer was resuspended in an equal volume of hypotonic buffer and centrifuged at $4000 \times g$ for 15 min. The final membrane preparation was stored in 3 ml of hypotonic buffer under nitrogen at 4 °C.

Separation of phospholipids from erythrocyte plasmalemmae. The membrane preparation was homogenized, on ice, in a glass on glass hand homogenizer for 15 min and diluted into an equal volume of fresh chloroform/methanol (3 : 1.5,

v/v). 0.01 M butylated hydroxytoluene in chloroform was added to the extraction medium to retard the peroxidation of the lipids. The homogenate was centrifuged at 2100 $\times g$ for 20 min and the upper phase was discarded. The lower organic phase was evaporated under a stream of nitrogen to a constant weight. The dried and weighted lipid material was dissolved in 50 μl of chloroform. Activated and washed Fisher Silica gel G rediplates were spotted with the 50 μl lipid solution containing approx. 1 μmol of P_i. The phosholipids were separated from each other using two-dimensional thin-layer chromatography. The phospholipids were separated with a chloroform/methanol/acetic acid mixture (40 : 20 : 4, v/v) in the first dimension. The plates were air dried for 5 min under nitrogen. The second dimension eluent solution consisted of chloroform/acetone/methanol/acetic acetic/water (20 : 8 : 4 : 4 : 2, v/v). The phospholipids were visualized with ultraviolet light after spraying with Rhodamine 6-G. The lipids were alternatively identified with ninhydrin reagent. Separated phopsholipid spots were removed from the silica gel plate and transferred to separatory funnels containing chloroform/methanol (10 : 10, v/v). 9 ml of distilled water was added. After shaking the lower layer was removed, transferred to vials, dried, sealed and stored under nitrogen at $-70\,^{\circ}C$.

Phospholipid quantification [17]. For all phosphorus assays, the glassware was acid washed. Phospholipid solutions were evaporated to dryness under nitrogen and 0.4 ml of concentrated H_2SO_4 was added. The phospholipids were charred by heating at 275 °C. After cooling, 5 to 10 drops of 30% H_2O_2 were added. The tubes were reheated to 100 °C for 20 min. Upon cooling this clear mixture, 8.8 ml of H_2O and 0.4 ml of 5% ammonium molybdate were added. Fiske and SubbaRow reagent was then added [18]. The solution was adjusted to a pH of 5.5 using 1 M NaOH. Then 0.5 g of sodium sulfite was added. If the tube turned yellow, sodium bisulfite was added. The tubes were covered with marbles, boiled for 7 min, and then cooled to room temperature. The solutions were analyzed spectrophotometrically at 660 nm for released inorganic phosporus.

Gas-liquid chromatography (method of Ferguson, K., personal communication). Methyl esters of fatty acids of phospholipids and sphingolipids were prepared by the following procedure. 1 ml of 0.5 M HCl in anhydrous methanol was added to dried lipid samples under nitrogen. The methanol was stored over anhydrous sodium sulfate. The sealed tubes were heated at 75 °C for 1 h under nitrogen and subsequently cooled. Then 2.0 ml of petroleum ether were added. The esters were extracted twice with petroleum ether, and the methyl ester extracts were combined. The solvent of the extracts was evaporated under nitrogen, and a few drops of benzene were added. The vials were stored under nitrogen.

The prepared methyl esters were spotted (2 μl) and eluted on a Varian AErograph Gas Chromatography (series 2100) using a H1-EFF-1-BP 15% diethyl glycol succinate column obtained from Applied Sciences. A calibration curve of relative retention times of various fatty acid methyl esters was prepared. The weights of eluted methyl ester peaks were obtained by cutting out observed peaks and weighing on a Perkin-Elmer AD_2 Automicrobalance. The amount of methyl ester applied to the column was calculated using a measured amount of methyl stearate as a standard.

Enzyme assays. The succinate dehydrogenase activity was determined according to the method of Earl et al. [19]. 5'-Nucleotidase activity was assayed by the method of Wong et al. [20]. Glucose-6-phosphatase activity was determined by the method of Nordlie et al. [21]. Protein concentration was estimated by the method of Lowry et al. [22]. Inorganic phosphate was quantified according to the Fiske ad SubbaRow procedure [18].

Data analysis. Data were compared by Student's *t*-tests. When appropriate, developmental data were fitted to a linear model using a least-squares regression analysis (Sokal and Rohlf) [23]. This model in conjunction with analysis of covariance was used to test for differences between normal and dystrophic regression lines (m and b). The correctional coefficient (r) was used to test the fit of the linear-regression model.

Results

Studies to determine the phospholipid content of the erythrocyte plasmalemmae from normal and dystrophic chickens

The question whether there might be quantita-

tive or qualitative differences in the plasmalemma phospholipid composition between normal and dystrophic chicken erythrocytes was investigated. Attesting to the purity of the membrane preparation, an increased specific activity was noted for the surface membrane marker, 5′-nucleotidase, in the final preparation while no enzyme activity was detected for the mitochondrial and microsomal membrane markers (Table I). There were no significant differences observed for marker enzyme specific activity values between normal and dystrophic chicken erythrocyte membrane preparations. Most of the succinate dehydrogenase activity was lost after the initial homogenization procedure while most of the glucose-6-phosphatase activity was lost during the hemolysis procedure. White cell contaminants were removed during successive isotonic rinsing. There was no significant change in the amount of protein in the membrane preparations from assay to assay.

Phospholipids were extracted from these membranes preparations. Fig. 1 depicts a typical two-dimensional thin-layer chromatograph identifying the relative positions of the separated phospholipids. This system completely separated sphingomyelin, phosphatidylcholine, lysophosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine, phosphatidic acid and neutral lipids. 69 percent of

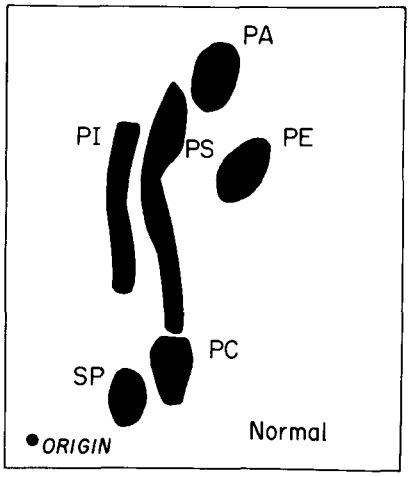

Fig. 1. Thin-layer chromatography. This representative two-dimensional thin-layer chromatograph identifies the relative positions of the separated phospholipids of a normal chicken erythrocyte plasura membranes. The captions represent: SP, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PS, phosphatidylserine; PA, phosphatidic acid.

TABLE I

PURIFICATION OF ERYTHROCYTE MEMBRANES

The activities of 5′-nucleotidase, succinate dehydrogenase and glucose-6-phosphatase were determined to test the relative purity of the membrane preparations. $N = 3$ 48-day ex ovo chickens for these purification assays. The values are presented as means ± S.D. Units of enzymatic activity: 5′-nucleotidase, one unit of activity is the amount of enzyme catalyzing the formation of 1 μmole of inorganic phosphate per h; glucose-6-phosphatase, one unit of activity is the amount of enzyme catalyzing the formation of 1 μmole of inorganic phosphate per h; succinate dehydrogenase, one unit of activity is the amount of enzyme catalyzing the oxidation of 1 μmole of succinate per h. n.d., not detected.

Preparation step	Specific activities in plasmalemma purification procedure (units/mg protein)					
	5′-Nucleotidase		Glucose-6-phosphatase		Succinate dehydrogenase	
	Dyst	Norm	Dyst	Norm	Dyst	Norm
Erythrocytes in NaCl	0.20 ± 0.03	0.21 ± 0.02	0.08 ± 0.05	0.10 ± 0.04	0.07 ± 0.03	0.08 ± 0.03
Sediment 1st wash	0.37 ± 0.04	0.38 ± 0.04	0.22 ± 0.03	0.20 ± 0.02	0.03 ± 0.01	0.03 ± 0.01
Sediment 4th wash	0.36 ± 0.02	0.37 ± 0.03	0.25 ± 0.03	0.25 ± 0.03	0.05 ± 0.01	0.06 ± 0.02
Sediment 1st hemolysis	0.39 ± 0.02	0.41 ± 0.02	0.29 ± 0.03	0.29 ± 0.03	0.07 ± 0.02	0.07 ± 0.01
Sediment 2nd hemolysis	0.42 ± 0.03	0.44 ± 0.03	0.32 ± 0.03	0.34 ± 0.03	0.03 ± 0.02	0.02 ± 0.02
Sediment 4th hemolysis	1.30 ± 0.11	1.26 ± 0.07	0.31 ± 0.07	0.31 ± 0.05	0.05 ± 0.02	0.04 ± 0.02
Homogenization	1.29 ± 0.04	1.33 ± 0.03	0.12 ± 0.03	0.11 ± 0.03	0.11 ± 0.05	0.08 ± 0.03
Sed 1st 6000 × *g* centrifugation	1.22 ± 0.17	1.20 ± 0.19	0.03 ± 0.03	0.02 ± 0.02	0.11 ± 0.04	0.10 ± 0.03
Sed 3rd 6000 × *g* centrifugation	1.41 ± 0.14	1.48 ± 0.05	n.d.	n.d.	0.01 ± 0.01	0.01 ± 0.01
Final preparation	1.81 ± 0.07	1.84 ± 0.06	n.d.	n.d.	n.d.	n.d.

the total lipid spotted was recovered as phospholipid inorganic phosphorus. In Table II, the phospholipid content of normal and dystrophic erythrocyte plasmalemmae is presented. The normal chicken erythrocyte plasmalemma was primarily composed of phosphatidyletholamine and phosphatidylcholine in an approximate molar ratio of 1 : 1. Sphingomyelin and phosphatidylserine also comprised a significant amount of the membrane preparation. Phosphatidylinositol and phosphatidic acid were minor components of the erythrocyte plasmalemmae. Lysophospholipids were not detected in either normal or dystrophic membrane preparations.

Concomitant with the onset of righting disability (Fig. 2), the dystrophic chicken erythrocyte plasmalemma has an increased concentration of phosphatidylserine and a decreased concentration of phosphatidylethanolamine compared with that of age and sex matched controls (Table II). The percentage of phosphatidylethanolamine in dystrophic membrane preparations was significantly different from control values ($P < 0.005$) subsequent to day 40 ex ovo. The concentration of phosphatidylserine in normal and dystrophic erythrocyte membranes were significantly different from each other at day 22 ex ovo ($P < 0.01$) and at days 40 through 158 ex ovo ($P < 0.005$). There

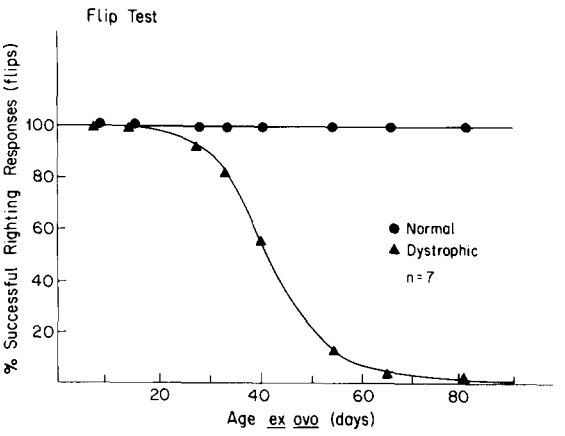

Fig. 2. Flip test. The abscissa represents age ex ovo and the ordinate represents percent successful righting responses for seven normal and dystrophic chickens. Each point represents the mean of seven experiments.

TABLE II

PHOSPHOLIPID CONTENT SUMMARY

The phospholipid content (mean ± 1 S.D.) is represented as the percent of total extracted plasmalemma phopsholipid recovered at several different ages of the experimental animals.

		Percent of total plasmalemma phospholipid; Day ex ovo								
		7	20	27	33	40	55	68	81	158
Phosphatidylcholine	N	28	20±2	28±5	26±4	27±2	26±2	28±2	24±2	26±3
	D	23	21±2	25±2	22±2	31±4	28±2	25±2	25±2	21±2
Sphingomyelin	N	21	14±2	16±2	19±3	19±3	14±2	18±2	21±2	13±3
	D	16	15±2	18±2	16±2	16±2	16±2	20±2	19±2	18±2
Phosphatidylethanolamine	N	16	23±3	19±2	30±4	34±5	28±2	30±2	28±3	31±3
	D	21	20±3	16±5	26±3	20±3	18±2	16±4	18±3	16±3
Phosphatidylserine	N	15	17±2	16±3	15±2	15±3	17±2	14±2	14±2	15±2
	D	17	21±3	23±2	20±3	19±2	22±2	20±2	21±2	21±2
Phosphatidylinositol	N	10	12±2	11±3	3±1	3±1	7±1	6±1	7±1	7±2
	D	11	11±2	9±2	6±2	5±2	6±1	5±1	5±1	8±2
Phosphatidic acid	N	9	13±2	10±3	5±2	2±1	6±2	4±1	6±1	6±1
	D	11	11±2	8±1	4±2	3±1	4±1	6±2	5±2	8±1
Unknown	N	–	–	–	–	–	–	–	–	–
	D	–	–	–	3±1	4±1	4±1	6±1	7±1	7±1

were no significant differences between normal and dystrophic values for any of the other isolated phospholipids.

The data for phosphatidylserine and phosphatidylethanolamine content over time may not fit a linear function. However, if linear regression lines were fitted to the phosphatidylserine and phosphatidylethanolamine percentages in normal and dystrophic membranes, then the regression lines (slopes and intercepts) were significantly different from each other ($P < 0.005$). The correlation coefficient, r, ranged from 0.6 to 0.97 for all of the plots of phospholipid percentage versus developmental age. These data also have been calculated as μmoles phospholipid phosphorus per μg lipid spotted. Similar results were obtained.

Concomitant with the changing phosphatidylserine and phosphatidylethanolamine percentages was the observation of a distinct and separate phospholipid on TLC plates spotted with dystrophic membrane preparations only. Figs. 3a and 3b represent TLC plates from 250-day ex ovo dystrophic and normal erythrocyte plasmalemmae. Phospholipids were visualized with ninhydrin which reacts with the NH_2 groups of serine and ethanolamine. these phospholipids were also visualized in similar positions with Rhodamine 6-G. ON TLC plates, spotted with extracted dystrophic membrane, the additional phopsholipid runs adjacent to phosphatidylethanolamine. This phospholipid comprised 3% to 7% of the dystrophic membrane lipid phosphorus (Table II).

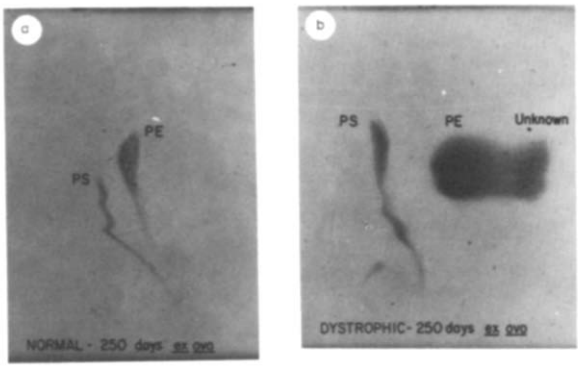

Fig. 3. Visualization of unknown phospholipid. These two-dimensional TLC plates of erythrocytes plasmalemmae preparation from 250-day ex ovo normal (a) and dystrophic (b) chickens were visualized with ninhydrin.

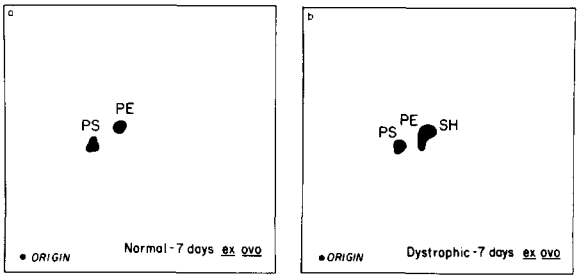

Fig. 4. TLC plates of normal (a) and dystrophic (b) 7-day ex ovo chicken erythrocyte plasmalemmae. These plates were sprayed with ninhydrin for visualization.

This compound was first visualized as a distinct spot at an age correlated with the onset of overt dystrophic symptoms (viz., righting disability). However, as early as day 7 ex ovo a 'shoulder' region was visualized on the phosphatidylethanolamine spot on dystrophic plates only (Figs. 4a and 4b). The precise identification and quantification of the 'shoulder' region was not undertaken at this age due to the indistinct separation of the regions as well as the low yield of phospholipid for analysis.

Studies to determine the fatty acid profile of the phospholipids of the erythrocyte plasmalemmae from normal and dystrophic chickens

The fatty acid composition of the isolated phos-

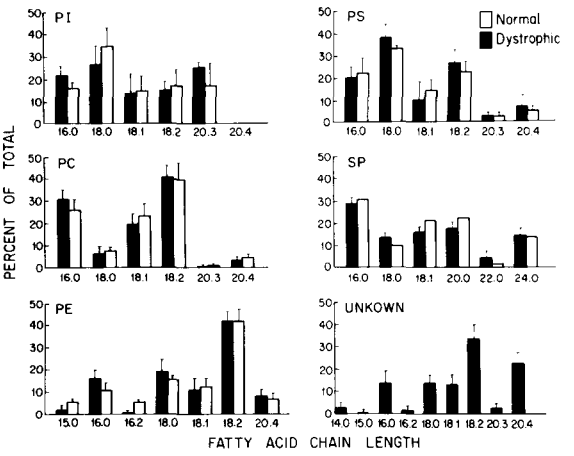

Fig. 5. Fatty acid composition. The abscissa represents fatty acid chain length and the ordinate represents percent of total fatty acid distribution. $N = 7$ for these studies of 139-day ex ovo normal and dystrophic chickens. (mean $\pm$ 1 S.D.).

pholipids from 139-day ex ovo normal and dystrophic erythrocytes was analyzed both quantitatively and qualitatively by gas-liquid chromatography (Fig. 5). All of the isolated phospholipids were composed of significant amounts of polyunsaturated fatty acid moieties, except for sphingomyelin which exhibited a predominantly saturated profile. The predominant fatty acid moiety for phosphatidylcholine and phosphatidylethanolamine was 18 : 2 compared with 18 : 0 for phosphatidylserine and phosphatidylinositol. There were no significant differences among the phospholipids in the major fatty acid patterns in normal and dystrophic erythrocyte membranes. Significantly lower concentrations of the minor fatty acids 15 : 0 and 16 : 2 were noted for phosphatidylethanolamine from dystrophic preparations compared with non-dystrophic erythrocytes ($P < 0.005$). A distinct fatty acid profile was noted for each phospholipid. Identical studies on samples from 350-day ex ovo chicken showed similar results.

Discussion

Alterations in phospholipid content are observed in dystrophic erythrocyte plasmalemmae compared with normal age matched controls. Specifically, dystrophic chicken erythrocyte plasmalemmae have higher concentrations of phosphatidylserine and lower concentrations of phosphatidylethanolamine than normal control chickens. Also, a distinct phospholipid is visualized to the right of phosphatidylethanolamine exclusively on TLC plates spotted with dystrophic erythrocyte membrane preparations. These structural anomalies of erythrocyte plasmalemmae may be related to a general defect in dystrophic membrane structure.

Before lipid separation on TLC, a relatively pure membrane preparation should be demonstrated. The final preparation of plasmalemmae is considered relatively pure when it is rich in 5′-nucleotidase activity and devoid of significant amounts of succinate dehydrogenase and glucose-6-phosphatase activity. It is inferred that the final membrane preparation used in these studies is devoid of significant mitochondrial and microsomal contamination. The increase in activity values that is observed for the microsomal and mitochondrial membrane markers in the early stages of purification may be deceiving. The loss of the major protein of the erythrocyte, hemoglobin, due to osmotic lysis and homogenization, may artificially raise the specific activity values for these markers. Alternately, a significant number of cells may remain intact during the initial lysis steps of the purification thereby increasing the specific activity values for these membrane markers (lower protein concentration of supernatant fluid).

The decreased content of phosphatidylethanolamine in dystrophic chicken erythrocytes can possibly be explained by the increased concentration of the unknown, ninhydrin positive, compound that is observed adjacent to phosphatidylethanolamine in the dystrophic plasmalemmae preparations. Also, the increased concentrations of the acidic phospholipid, phosphatidylserine, may be correlated with the decrease in phosphatidylethanolamine concentration since serine and ethanolamine can be interchanged between phospholipids through either a decarboxylase or a serine transferase [24].

Chalovich and Barany [25] have identified through NMR studies an embryonic component in dystrophic chicken muscle and erythrocyte membrane preparations: serine-ethanolamine-phosphodiester. These observations deserve further consideration, especially since our studies not abnormalities in the content of membrane phospholipids containing these polar headgroups. The increased concentrations of phosphatidylserine in dystrophic erythrocyte plasmalemmae which are reported here partially may be due to the high levels of serine ethanolamine phosphodiester in dystrophic membranes. This phosphodiester may be serving as a precursor storage site of serine moieties for phosphatidylserine synthesis. This is especially possible since Chalovich and Barany [25] have suggested that the function of the phosphodiester is not related to phosphatidylserine degradation.

The phospholipid anomalies observed in dystrophic erythrocyte membranes also can be rationalized by a defect in phospholipid turnover rate. An increased turnover rate for phosphatidylethanolamine in dystrophic erythrocytes can account for the observed decreases in phos-

phatidylethanolamine concentration in dystrophic erythrocyte plasmalemmae. This decreased turnover may be due to an anomaly in the enzymes of phosphatidylethanolamine metabolism, either an increase in phosphatidylethanolamine degradation (phospholipase, decarboxylase) and/or a decrease in phosphatidylethanolamine synthesis (cytidine diphosphate-ethanolamine phosphotransferase or serine transferase). Similarly, a decreased turnover rate for phosphatidylserine in dystrophic erythrocytes can account for an elevated concentration of phosphatidylserine in dystrophic erythrocyte membranes.

The possible functional effects of decreased phosphatidylethanolamine and increased phosphatidylserine concentration in dystrophic erythrocyte membranes are not determined in this study. However, the acidic phospholipids are thought to play a role in the activity of certain membrane bound enzymes, particularly adenylate cyclase and $(Na^+ + K^+)$-ATPase [26] which has been shown to have peculiar properties in the dystrophies [4,27]. Moreover, changes in membrane fluidity, manifested either through polar head group specificity or fatty acid profile can also have significant effects on the enzymes embedded within the membrane

Erythrocyte plasmalemmae from dystrophic chickens have a decreased fluidity compared with those from normal chickens [2]. The fatty acyl components of normal and dystrophic erythrocyte plasmalemma phospholipids do not offer a conclusive explanation for possible fluidity changes in the chicken since no gross differences in the major phospholipids are noted between these preparations. However, the fatty acid pattern of phosphatidylethanolamine from dystrophic chicken erythrocyte membranes is characterized by a decrease in the amount of small chain length (15 : 0, 16 : 2) fatty acids. This fatty acid decrease could increase the viscosity of the membrane since small chain length fatty acids create disorder in membranes by reducing Van de Waals and hydrophobic interactions. Phosphatidylethanolamine may decrease membrane fluidity locally due to its ethanolamine per head group since proton and phoshorus NMR of sonicated lipid dispersions of phosphatidylcholine and phosphatidylethanolamine suggest that the molecular motions of phosphatidylethanolamine are more restricted than those of phosphatidylcholine [28]. The actual contribution of each of the phospholipid anomalies to the overall decrease in fluidity in chicken dystrophy or changes in the fluidity of local membrane environments has yet to be studied. Also, the characterization of the unidentified, ninhydrin positive, phospholipid adjacent to phosphatidylethanolamine and its possible interactions with other membrane components should be investigated.

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