THE EFFECT OF DIMETHYL SULFOXIDE ON HUMAN ERYTHROCYTE MEMBRANE

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ABSTRACT.—*Isolated human erythrocyte membranes were submitted to sonication in the presence and absence of dimethyl sulfoxide. Solubilized protein fractions containing high sialic acid and low lipid content were obtained. The possible role of DMSO as a cryoprotective agent in a membrane system was discussed.*

The human erythrocyte membrane has been the subject of intensive research because of its ready availability and ease of isolation. Knowledge of the precise arrangement of its components may open the way for treatment of blood diseases such as hereditary elliptocytosis and hereditary spherocytosis, which are suspected to be caused by membrane defects. The structure of erythrocyte membranes may also be common to that of other biological membranes. It is therefore hoped that other diseases may be explainable in terms of membrane activity.

Adequate methods of solubilization of the membrane components must be developed before the structure of the membrane can be elucidated. Several attempts to develop suitable procedures have been made (Maddy, 1970). These include organic solvent extractions (butanol, 2-chloroethanol, phenol, aqueous pyridine and pentanol), detergent solubilization (cholate, deoxycholate, sodium dodecyl sulfate and Triton X100), as well as hydrogen bond-breaking reagents (urea and guanidine hydrochloride). However, these reagents either lack specificity in solubilizing a particular component, forming complexes with the membrane components, or denature proteins. Therefore, it is important to explore the potential of other reagents for the specific removal of membrane components. For example, dimethyl sulfoxide (DMSO) has been known for its usefulness in the solubilization of glycogen (Whistler and DeMiller, 1962) and selective extraction of lipopolysaccharide from the outer membrane of Gram-negative bacteria (Adams, 1967).

It would be of interest to determine the chemical nature of the components extractable from human erythrocyte membranes by dimethyl sulfoxide. This report represents a study of the extraction procedures and the chemical analyses of the extracts as well as the residues.

MATERIALS AND METHODS

Hemoglobin-free human erythrocyte membranes were isolated from outdated blood (Peoria Red Cross, Peoria, Illinois) according to the procedure of Dodge, *et al.*, 1963. The cells were washed three times in isotonic 310 ideal milliosmolal phosphate buffer, pH 7.4, lysed overnight in hypotonic 20 ideal milliosmolar phosphate buffer and washed with hypotonic buffer until free of hemoglobin.

After dialysis and lyophilization a 200 mg sample of membrane was added to 50 ml of 0.1 M phosphate buffer, pH 7.4, and stirred to homogeniety for 1-2 hours. An equal volume (50 ml) of DMSO was slowly added, stirred to homogeniety for 1 hour, and sonicated (Branson Sonicator Model W140D) in 50 ml aliquots at 4° C at 60 watts for 6 minutes. Centrifugation was performed at 20,000 x g for 20 minutes at 4° C after the sonicates were combined. The supernate was de-

anted, and the precipitate was reonstituted for 2-3 hours in 25 ml f 0.1 M phosphate buffer, pH 7.4. n additional portion of DMSO 25 ml) was slowly added to the spension; sonication and centrifuon were executed as before. The wo combined supernates (Fracon S) and the reconstituted precitate (Fraction P) were dialyzed gainst distilled water for one week nd lyophilized.

A control sample was treated xactly as described above, except n equal volume of 0.1 M phoshate buffer, pH 7.4, was added to ne buffer-membrane suspension in lace of the equal volume of DMSO. 'he supernate and precipitate of nis treatment were recovered as IS and NP, respectively.

Protein (Lowry, *et al.*, 1959), hexsamine (Rondle and Morgan, 955), hexoses (Koehler, 1952), silic acid (Warren, 1959), and phoshorous (Bartlett, 1959) were deermined. Total lipids were exracted with chloroform:methanol 2:1 v/v) in a Soxhlet apparatus nd determined gravimetrically.

Results and Discussion

Dimethyl sulfoxide (DMSO), an protic solvent, is used in biologial systems to protect cells against reezing and radiation damage (Farant, 1965; Chang and Simon, 1968). ne of the hypotheses to explain ne cryoprotective and radio-proective action of DMSO assumes nat this solvent prevents changes n the cell's lipoprotein membranes nd stabilizes lipoprotein complexes Keysary and Kohn, 1970). Five to en percent DMSO is widely used s an additive for the protection of nimal cells during freezing storge. However, when continuously resent, these concentrations might e toxic. It seems therefore paraoxical that a toxic substance should e protective. A similar situation occurs in the bacterial endotoxin systems, which are toxic over high concentration and protective at low concentrations. Furthermore, it is puzzling that a reagent known to extract carbohydrates (Whistler and DeMiller, 1962; Adams, 1967) would serve as a stabilizing agent for the membrane system which is known to consist of protein, lipids and carbohydrates (Bakerman and Wasemiller, 1967).

In experiments reported here, a high concentration of DMSO (50%) in a buffered solution was used to extract human erythrocyte membranes. DMSO extraction along with sonic treatment was found to solubilize 28.7% while sonic treatment alone solubilized 23.6%. In both cases, the ratio of the amount of residue to amount solubilized was approximately 2:1 (Table 1 and 2). Since the presence of DMSO did not affect the relative ratio of residues to solubilized material, it appears that sonication alone was responsible for the extraction efficiency. In order to determine the chemical nature of the fractions, chemical analyses were performed (Table 1 and 2). In both cases significant amounts of total carbohydrates (15%), especially sialic acids (5%) was found in the solubilized fractions. On the other hand, there was a slightly larger amount of total free lipids in the residues (60%) than in the solubilized fractions (45%). There was no significant difference in the amount of proteins (43%) in the residues which have a similar protein composition as the starting intact membranes. The sialic acid-rich fractions (Fractions S and NS) resemble those prepared by aqueous pyridine extraction (Blumenfeld, 1968) and by pronase treatment (Ohkuma and Furuhata, 1969) of human erythrocyte membranes. From these studies, it seems that there are two types

TABLE 1. Chemical Composition of Human Erythrocyte Membrane Fractions After Sonication Treatment in the Presence of Dimethyl Sulfoxide

Fractions	S	P	Intact Membrane	
			Experimental	Literatu
Yield (%)	28.7	49.6	0.7608*	....
Protein (%)	34.9	42.2	46.1	55.0**
Total Lipid (%)	50.0	61.0	37.2	35.0**
Phosphorous (%)	1.40	1.29	0.90	1.10***
Total Carbohydrate (%)	15.19	11.28	9.49	10.0**
Hexosamine (%)	5.00	3.92	3.00	
Sialic Acid (%)	5.35	2.02	2.57	
Hexoses (%)	4.84	4.34	3.92	

*Number of grams from 600 ml blood
**Bakerman, *et al.*, 1967
***Lauf and Poulik, 1968

TABLE 2. Chemical Composition of Human Erythrocyte Membrane Fractions After Sonication

Fractions	NS	NP	Intact Membrane	
			Experimental	Literatu
Yield (%)	23.6	49.8	0.7608*	....
Protein (%)	33.0	44.1	46.1	55.0**
Total Lipid (%)	45.0	60.0	37.2	35.0**
Phosphorous (%)	1.46	1.08	0.90	1.10***
Total Carbohydrate (%)	15.89	9.32	9.49	10.0**
Hexosamine (%)	4.90	3.82	3.00	
Sialic Acid (%)	5.10	1.70	2.57	
Hexoses (%)	5.89	3.80	3.92	

*Number of grams from 600 ml blood
**Bakerman, *et al.*, 1967
***Lauf and Poulik, 1968

of proteins in the erythrocyte membrane, one is high in sialic acid and low in lipid; the other low in sialic acid and high in lipid. In both the aqueous pyridine and pronase preparations, the fractions rich in sialic acids bear antigenic determinants of the MN blood group system. It is not known if the sialic acid-rich fractions isolated by DMSO treatment would share this immunological property.

It is surprising that DMSO failed to extract much free lipid. It would be expected that sonication alone in an aqueous medium would be less effective and DMSO, being an aprotic organic solvent, more effective in removing lipid materials. O the other hand, this unique prop erty of DMSO may explain the pos sible stabilization effect on the lipo protein complexes of the membran

It has been reported (Rosenber and McIntosh, 1968) that sonica tion does not alter the molecula membrane structure. The solubil zation effect by sonication was sug gested to be caused by the disir tegration of the total membran system and reconstitution to sma unit-membrane pieces which di not sediment under high centrifu gation force. These solubilized unit membranes were shown to hav similar chemical composition as th

intact membranes. Our results are not consistent with their findings. Similar to the treatment in the presence of DMSO, sonication alone was able to release a sugar-rich moiety (Fraction NS). The exact nature of the solubilized material in the presence and absence of DMSO may not be known until their homogeneity is established by column fractionation or polyacrylamide disc electrophoresis. These solubilized fractions may well be a complex (or complexes) of lipids, proteins, glycoproteins and glycolipids. One thing is certain, however, that there is an easily releasable sialic acid-rich moiety in the human erythrocyte membrane.

Although DMSO extraction did not provide a clear-cut selectivity for any particular components of the erythrocyte membrane, the inability of DMSO to remove lipid to any great extent does not contradict with the suggestion that membrane lipids may be the site or sites for freezing injury to cellular systems (Livne, 1969) which could be protected by low concentrations of DMSO.

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