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Oxygen Solubilization in Egg Lecithin Dispersed in Distilled Water and Physiological Electrolyte Fluids

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Abstract □ Gaseous oxygen solubilization in egg lecithin dispersed in distilled water, saline, and a multi-ion physiological electrolyte solution was determined and compared to controls deficient in egg lecithin. Significant oxygen solubilization occurred in the presence of egg lecithin. Oxygen solubilization was significantly greater in saline and in the multi-ion physiological electrolyte solution than in distilled water.

Keyphrases □ Solubilization—of oxygen in egg lecithin dispersed in distilled water and physiological electrolyte fluids □ Lecithin, egg—dispersions in distilled water and physiological electrolyte fluids, oxygen solubilization □ Pulmonary surfactant model systems—egg lecithin dispersions in distilled water and physiological electrolyte fluids, oxygen solubilization □ Model systems, pulmonary surfactant—egg lecithin dispersions in distilled water and physiological electrolyte fluids, oxygen solubilization □ Oxygen—solubilization in egg lecithin dispersions in distilled water and physiological electrolyte fluids

Several studies (1–5) demonstrated that respiratory disease syndrome[1] is a direct result of a pulmonary surfactant deficiency. Mammalian lung surfactant was shown to be primarily dipalmitoyl phosphatidylcholine (I) (6–8). Since I also is the major constituent of egg (*Gallus domesticus*) lecithin (9), egg lecithin dispersions serve as convenient and relatively inexpensive pulmonary surfactant model systems.

[1] Hyaline membrane disease.

Micellar oxygen solubilization in lung surfactant was proposed (10) as a mechanism for oxygen transposition at the alveolar membrane. Other studies (11–14) demonstrated the ability of lung surfactant to solubilize oxygen and other nonpolar gases.

The effect of the presence of electrolytes at physiological concentrations on oxygen solubilization in aqueous egg lecithin dispersions is reported here.

EXPERIMENTAL

Glass reaction vials were cleaned ultrasonically[2] in 2% aqueous detergent[3], rinsed three times with tap water, and rinsed three times with deionized, glass-distilled water[4]. The vials were air dried in a ventilated oven at 200°.

Egg lecithin[5] was weighed accurately to yield 50-ml samples of 0.25, 0.50, 0.75, and 1.00% (w/v) phospholipid in normal saline[6], physiological electrolyte solution[7], and deionized, glass-distilled water. A magnetic

[2] Cole Palmer Co., Chicago, Ill.
[3] Alconox, New York, N.Y.
[4] Deionized through an exchange resin deionizer (Continental Water Service, Oklahoma City, Okla.) and then glass distilled.
[5] Lot 12073, United States Biochemical Corp., Cleveland, Ohio.
[6] Travenol Laboratories, Deerfield, Ill.
[7] Normosol-R, Abbott Laboratories, North Chicago, Ill. Contains sodium, potassium, magnesium, chloride, and bicarbonate ions in isotonic aqueous solution.

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Table I—Three-Factor Analysis of Variance with Repeat Measures for Oxygen Solubilization in Egg Lecithin Dispersions Excluding Controls

Source of Variation	*df*	*SS*	*MS*	*F*[a]
Media	2	15.0288	7.5144	60.41
Concentration	3	4.5492	1.5164	12.19
Media × concentration	6	12.3654	2.0609	16.57
Subjects (G)	60	7.4632	0.1233	—
Time	4	1012.0422	253.0106	10,120.42
Time × media	8	48.9936	6.1242	244.97
Time × concentration	12	2.4318	0.2027	8.11
Time × media × concentration	24	3.4116	0.1422	5.69
Time × subjects (G)	240	5.9930	0.0250	—

[a] $p < 0.01$.

Table II—Three-Factor Analysis of Variance with Repeat Measures for Oxygen Solubilization in Egg Lecithin Dispersions Including Controls

Source of Variation	*df*	*SS*	*MS*	*F*[a]
Media	2	10.771	5.385	26.28
Concentration	4	351.825	87.956	429.26
Media × concentration	8	17.869	2.233	10.90
Subjects (G)	75	15.371	0.205	—
Time	4	827.529	206.882	9577.87
Time × media	8	40.475	5.059	234.21
Time × concentration	16	187.558	11.722	542.69
Time × media × concentration	32	12.129	0.379	17.55
Time × subjects (G)	300	6.484	0.022	—

[a] $p < 0.01$.

stirring bar was included in each sample vial before addition of the dispersion medium. Each vial was sealed with a rubber septum and an aluminum crimp seal and then was flushed with ultrapure (99.99%) humidified nitrogen[8]. Accurately measured volumes of the dispersion media were added after an equivalent volume of nitrogen gas was withdrawn from the vial. Each vial then was stirred gently on a magnetic stirring plate until homogeneous dispersion was effected.

Ten milliliters of nitrogen gas was withdrawn accurately from each vial, and an equivalent volume of ultrapure oxygen[9] was added with gas-tight gas transfer syringes[10]. While the dispersions were stirred gently on the magnetic stirring plate, 10-μl samples of the gaseous phase were withdrawn at 0, 24, 48, 96, and 120 hr.

Vials of normal saline, physiological electrolyte solution, and distilled water were prepared deficient in egg lecithin to serve as controls.

Each egg lecithin sample and its corresponding control sample were prepared and analyzed in triplicate, and each experiment was done in duplicate.

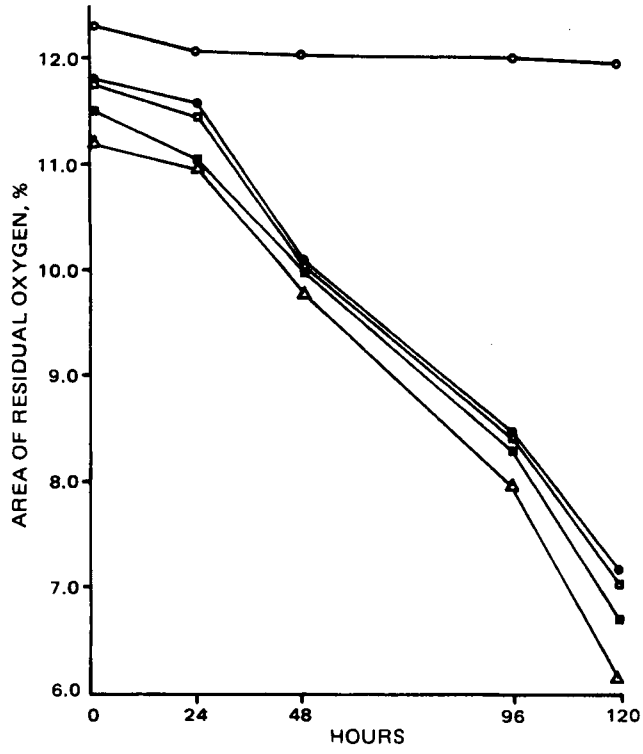

Figure 1—*Effect of egg lecithin concentration in normal saline on the extent of oxygen solubilization with time. Key: ○, normal saline (control); ●, 0.25% egg lecithin; □, 0.50% egg lecithin; ■, 0.75% egg lecithin; and △, 1.00% egg lecithin.*

[8] Union Carbide Corp., New York, N.Y.
[9] Ultrahigh purity, Union Carbide Corp., New York, N.Y.
[10] Models 1710 SN and 1020 LTN, Hamilton Syringe Co., Reno, Nev.

Analysis of the residual oxygen at the indicated times was accomplished by GLC[11]. The nitrogen–oxygen mixture was resolved on a molecular sieve[12] with thermal conductivity detection[13]. The peak area as the percent of detector response for oxygen was calculated and recorded with an electronic integrator[14].

Helium[8] was the carrier gas at 21 ml/min. The inlet, detector, and column temperatures were 100, 100, and 50°, respectively.

Since the sealed experimental and control vials were flushed with nitrogen to an open atmosphere and an equivalent volume of nitrogen gas was withdrawn when oxygen was added, the pressure in the closed system was equal to atmospheric pressure during sample preparation. Minor pressure effects due to the withdrawal of 10-μl gas samples at the indicated times were regarded as insignificant. Furthermore, any manipulative error introduced was offset in the statistical analysis because the control samples were treated the same as the experimental samples.

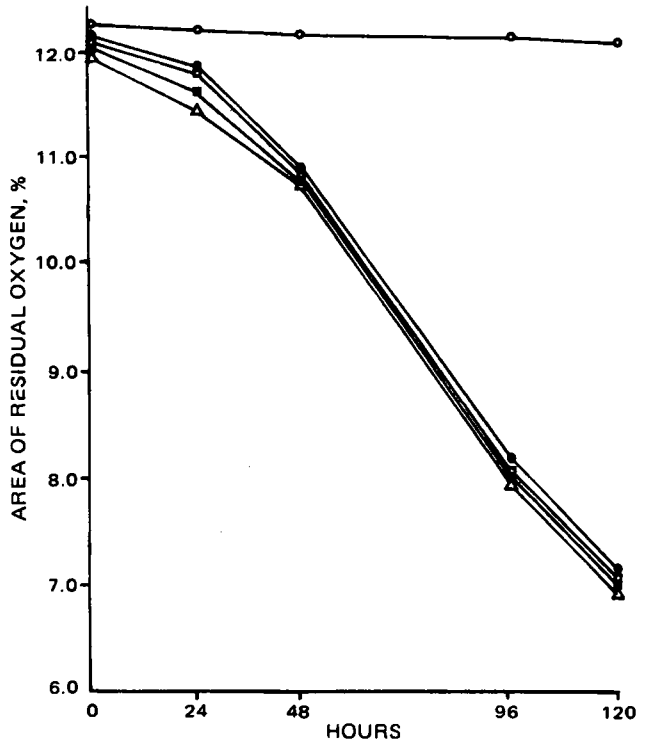

Figure 2—*Effect of egg lecithin concentration in a multi-ion physiological electrolyte fluid on the extent of oxygen solubilization with time. Key: ○, multi-ion physiological electrolyte solution (control); ●, 0.25% egg lecithin; □, 0.50% egg lecithin; ■, 0.75% egg lecithin; and △, 1.00% egg lecithin.*

[11] Model 2300 dual-column gas–liquid chromatograph, Bendix Corp., Ronceverte, W. Va.
[12] Molecular sieve 5-A, 80–100 mesh, and a 183-cm stainless steel column, Alltech Associates, Arlington Heights, Ill.
[13] Type 2300, four element, Bendix Corp., Ronceverte, W. Va.
[14] CDS IIIC, Varian Associates, Palo Alto, Calif.

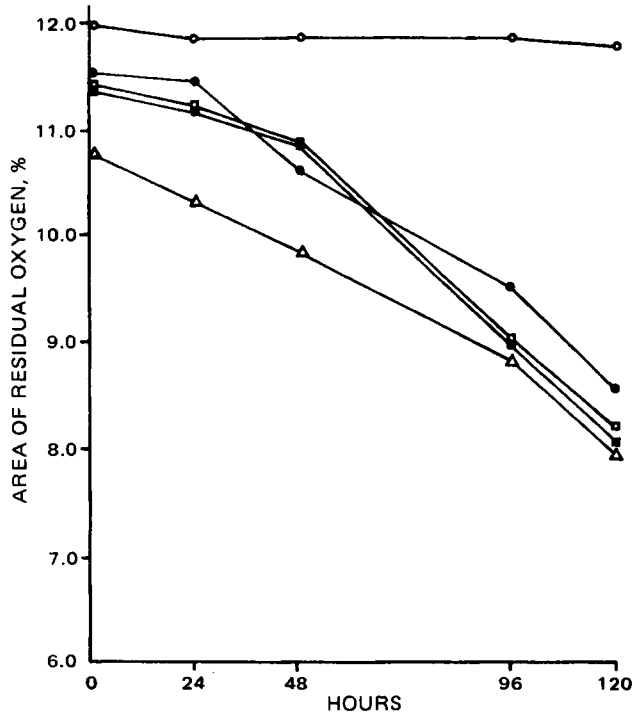

Figure 3—*Effect of egg lecithin concentration in distilled water on the extent of oxygen solubilization with time. Key:* ○, *deionized distilled water (control);* ●, *0.25% egg lecithin;* □, *0.50% egg lecithin;* ■, *0.75% egg lecithin; and* △, *1.00% egg lecithin.*

RESULTS AND DISCUSSION

Figures 1–3 indicate that, in comparison to the controls, considerable oxygen solubilization occurred in aqueous egg lecithin dispersions. Furthermore, differences in the amount of gaseous oxygen solubilized can be demonstrated for all three aqueous media used, with the multi-ion physiological electrolyte fluid and saline exhibiting the greatest solubilization. Statistical analysis of the data (Tables I and II) utilizing a three-factor analysis of variance indicated statistically valid differences in gaseous oxygen solubilization among the media employed, the egg lecithin concentration, and time.

Table II presents the statistical data including the control samples, while Table I excludes the controls. This contrast was done to identify the greatest variance ratio components. The incubation time had the greatest influence on oxygen solubilization, followed by the medium employed and then the egg lecithin concentration. This egg lecithin concentration effect, as determined statistically, is not surprising because solubilization requires only that micelles be present. Apparently, the egg lecithin concentration range used (0.25, 0.50, 0.75, and 1.00%) was above the critical micelle concentration for the surfactant in the media.

The presence of electrolytes can affect the size of surfactant micelles by forcing surfactant molecules into micellar units similar to the way in which electrolytes can be used to dehydrate or salt out sparingly soluble organic molecules from water.

The diffusional aspect of oxygen transposition was the rate limiter because the effect of time on oxygen transposition can be demonstrated statistically to be the predominant factor controlling oxygen solubilization.

This study indicated that, in the development of synthetic, natural, or semisynthetic lung surfactant for treatment of respiratory disease syndrome and other pulmonary surfactant diseases, the medium employed may overshadow the particular surfactant used. Further study with the egg lecithin pulmonary surfactant model system is warranted.

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