renal clearance of bethanidine declined continually with time in the initial absorptive phase during which there were both a rise and a fall in blood concentration. Therefore, the change in renal clearance was not apparently related to the blood concentration of the drug. In addition, the reduction in renal clearance occurred in the absence of any noticeable hypotensive response.

In conclusion, this report clearly demonstrates the desirability of simultaneous measurements of drug levels in both blood and urine when studying the pharmacokinetics of drugs. If concomitant plasma or blood measurements are not available and if the constancy of renal clearance cannot be assured, information derived from urinary excretion data should be interpreted with caution.

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Oxygen Solubilization by Lung Surfactant

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Abstract □ To illustrate the concept of solubilization as a possible mode of gas transport in biological systems, dog lung surfactants in varying concentrations were tested for their ability to solubilize oxygen. The degree of gas solubilization was determined by GC, using a modified tonometer as an absorption chamber. The concentration of surfactant was found to be an essential factor for gas solubilization. Surfactant concentration above the CMC yielded anomalously high gas absorption. Solubilization of the gas is thought to occur by a partitioning effect into the interior of surfactant micelles.

Keyphrases □ Surfactants, lung—oxygen solubilization at various surfactant concentrations □ Solubilization, oxygen—by various concentrations of dog lung surfactant

Throughout this study, the theory of gas uptake was developed from a reasonably well-established concept, *i.e.*, that of solubilization. Since solubilization is frequently applied to pharmaceutical systems, it is natural for this pharmaceutical background to allow correlation of many biological occurrences through similar mechanisms.

Solubilization is defined as the "spontaneous dissolution of normally water-insoluble substances by an aqueous solution of surfactant." The surfactant molecule is amphiphilic, possessing both a nonpolar (hydrophobic) portion and a polar (hydrophilic) portion which exist in solution as individual molecules in very dilute concentrations. With an increase in surfactant concentration above the critical micelle concentration (CMC), all surface-active agents have the ability to form micelles.

A micelle can be defined as an aggregate of surface-active agents acting as a unit. The basic physical characteristics of surface-active agents, which are present during and after the formation of micelles, are that they lower surface tension in dispersion, produce a wetting effect, are strongly adsorbed to hydrophobic surfaces, and can solubilize normally insoluble substances.

In this study, varying concentrations of dog lung surfactant were analyzed for oxygen-absorbing or solubilizing characteristics to illustrate the concept of solubilization as a possible mode of gas transport in biological systems, as proposed by Ecanow *et al.* (1–3). A great deal of information is available regarding the nature and composition of mammalian alveoli based on histochemical and extraction studies (4–11). Analyses of lung extracts have indicated that lecithin (phosphatidyl choline) depicts the characteristic surface-active properties of lung surfactant and that dipalmityl lecithin is the essential lung surfactant (12, 13).

EXPERIMENTAL

Gas solubilization was analyzed by GC. A modified tonometer, constructed of glass and fitted with rubber septums for the introduction and extraction of gas samples, was used as an absorption chamber (14, 15).

Gas syringes were used for transfer of the gas from the preparation tonometer to that containing the gas uptake sample. These syringes were fitted with 22-gauge, 6.35-cm (2.5-in.) needles. Gastight syringes[1] (100 and 250 μl), fitted with Teflon hub needles [24 gauge, 3.8 cm (1.5 in.)], were used for removal of gas analysis samples from the second tonometer and for injection of the samples into the gas chromatograph.

A gas chromatograph[2] equipped with a thermal conductivity detector was utilized to follow gas uptake. Helium, used as the carrier gas, was maintained at a flow rate of 40 ml/min. Operating temperatures were as follows: injection, 172°; column, 135°; internal detector, 137°; duct, 204°; outlet, 252°; and external oven, 232°. Stainless steel columns[3] used for analysis of gas uptake were 1.8 m × 0.64 cm (6 ft × 0.25 in.), packed with molecular sieves (5A).

Gas analysis samples were removed at intervals of approximately 1 min for 20 min; this time length had been determined as sufficient for data evaluation. The studies were conducted at ambient temperature and atmospheric pressure.

The lung surfactant solution was first warmed in a water bath at

[1] Hamilton.
[2] WCLID 1670, Warner-Chilcott Laboratories, Richmond, Calif.
[3] HCl Scientific Inc., Rockford, Ill.

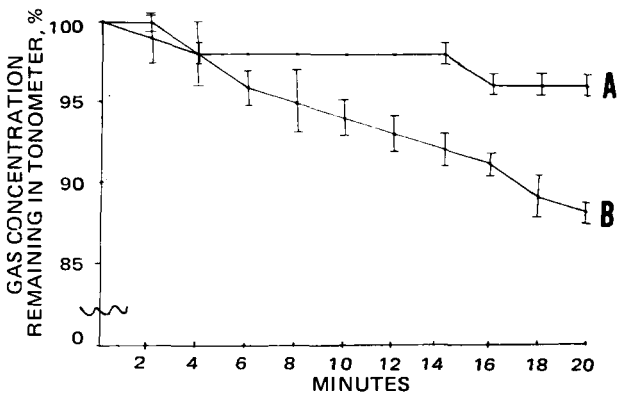

Figure 1—*Oxygen gas sorption by dog lung surfactant. Key: A, dilute lung surfactant in normal saline; and B, concentrated dog lung surfactant.*

40° to facilitate the escape of any dissolved gases, and the "degassed" lung surfactant solution was then placed in the sample tonometer. Helium was then passed through the tonometer to "clear" the atmosphere.

Oxygen was obtained in the form of oxygen USP. To allow for consistent transfer procedures, the oxygen was stored in a 300-ml tonometer at ambient temperature and atmospheric pressure. Since the columns used for oxygen detection in the chromatograph were also sensitive to nitrogen, tonometers were completely cleared with helium before being used for oxygen storage. This sensitivity to nitrogen was beneficial in that any leakage or contamination could be detected by the presence of a nitrogen peak.

Dogs were sacrificed by intravenous injection of pentobarbital sodium. Crude extracts of lung surfactant were prepared by the Bondurant–Miller (16) method, which produced a bulk liquid solution. A 0.9% sodium chloride solution, equivalent to 5–6 ml/g of lung, usually about 175 ml in total, was introduced through a tracheal cannula. From 70 to 80% of the volume was recovered from the tracheal (or bronchial) effluent. The liquid was retained in the lung for 10 min and then gently rinsed back and forth into a syringe five to 10 times. The solution became slightly turbid and, if allowed to stand, persisted in turbidity without formation of any significant precipitate except for a few mucoid strands. If centrifuged, however, the solution cleared and a distinctive precipitate was formed.

The lung extract was then divided into two equal parts. One portion was designated "dilute lung surfactant"; the remainder of the solution was concentrated to 20 ml by directing a jet of compressed air across the surface of the solution. This concentrate was then designated "concentrated dog lung surfactant."

RESULTS AND DISCUSSION

Figure 1 illustrates oxygen absorption obtained from studies on dilute lung surfactant in normal saline and concentrated dog lung surfactant. In original studies, the tonometer gas concentration (microliters per milliliter) in the vapor phase above the liquid sample *versus* the time (minutes) was followed graphically. For presentation purposes, graphic data were arranged to indicate the presence of the gas concentration remaining in the tonometer *versus* time (minutes). Results (Table I) indicate that oxygen absorption was negligible with distilled water and dilute surfactant in normal saline. The degree of oxygen solubilization with concentrated lung surfactant was significant, suggesting that solubilization of oxygen is mediated by micelle formation.

The oxygen gas absorption, found only in concentrated dog lung surfactant, indicates that micellar solubilization of oxygen may depend on the degree of micelle development in the surfactant solution. The dilute samples, ostensibly exhibiting little or no micelle development, showed insignificant gas absorption.

In previous studies (1, 2), a two-stage mechanism was proposed for molecular transport through an alveolar membrane across an aqueous barrier containing surfactants. The ability of the medium

Table I—Statistical Statement: *t* Values Based on Comparison between Degree of Oxygen Solubilization by Diluted Dog Lung Surfactant and Concentrated Dog Lung Surfactant Solutions

Minutes	$t_{[4]}$
0	0.00[a]
2	1.06[a]
4	0.32[a]
6	3.46[a]
8	3.00[a]
10	6.93[a]
12	8.66[b]
14	8.50[b]
16	12.72[b]
18	10.73[b]
20	16.97[b]

[a] Not significant. [b] $p < 0.001$.

interphase to concentrate the surfactant molecules to the aggregation point results in formation of micelles. A continued concentration increase unites the micelles to form large multicellular "films" at the alveolar membrane surface. These micellar films readily solubilize various nonpolar molecules.

In the second stage of the process, the interface is expanded to the point of micelle desegregation. The medium then consists of individual surfactant molecules with smaller micelles dispersed in the aqueous solution. The nonpolar gases are released to the interphase, and the essentially nonpolar molecules are readily soluble in portions of the alveolar membrane. The driving force for aggregation and dispersion of surfactant molecules in the lung lining is the expansion and contraction of the membrane surface during inhalation and exhalation (9).

Further ramifications of these findings are being studied.

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