Surface-active Material in Fetal Lung Fluid Concentrated by Foaming

By
Göran Enhörning,* Tetsuro Fujiwara** and Forrest H. Adams

From the Department of Pediatrics, School of Medicine, University of California, Los Angeles

(Received for publication, July 28, 1964)

A fluid flowing from the trachea of fetal lambs was collected and its surface active components were concentrated by foaming. The foam showed a greater concentration of active phospholipids and a greater surface stability than did the crude lung fluid. The residuum after foaming had less phospholipids and less surface stability than the crude lung fluid.

A fluid has been demonstrated to flow from the trachea of fetal lambs1,2). By studies with the modified Wilhelmy balance the fluid has been found to have a very low surface tension when the surface area is compressed to 20% of its maximal size3). These findings are of particular interest since the fluid originates from the lungs and is aspirated with initial aeration. It is likely that the surface activity of this fluid may be of importance in stabilizing the alveoli. The surface-active material in the lung is thought to be a lipoprotein with a phospholipid as the major active component4,5,6). It has been demonstrated that fetal lung fluid contains an appreciable amount of phospholipids3). It has also been shown that the active components of this material are lecithin and sphingomyelin7).

According to Gibb's law, a surface-active material is concentrated in an air-liquid interface8). The foam produced by bubbling nitrogen through crude lung fluid is therefore likely to contain a greater concentration of surface-active material. The results of concentrating surface-active material from fetal lung fluid according to this principle are presented here. Crude fetal lung fluid, the foam obtained from it, and the residuum have been analyzed for active phospholipid components and surface properties.

* Present address: Department of Obstetrics and Gynecology, Sabbatsberg Hospital, Karolinska Institute, Stockholm, Sweden.
** Fujiwara, T., 武居敏郎, Present address: Department of Pediatrics (Prof. Ts. Arakawa), Faculty of Medicine, Tohoku University, Sendai, Japan.
MATERIAL AND METHODS

Fetal lung fluid was obtained from lambs in the manner previously described\(^1\). Foam was produced and collected according to the method of Schütz\(^9\). Crude fluid, foam and the residuum were freeze-dried by a lyophilizer. Lipids were then extracted with chloroform: methanol (2:1, v/v) and purified according to the method of Folch et al.\(^10\)

Separation and assay of phospholipid components were accomplished with thin-layer chromatography\(^7\).

The method\(^11\) of evaluating surface properties was to record the pressure gradient between a sample liquid and a bubble in this liquid. Since the bubble communicates with atmosphere, the pressure gradient \(\Delta P\) is known by recording the negative pressure in the liquid. From the formula of Laplace, \(\Delta P = 2\gamma/r\), the surface tension (\(\gamma\)) can be calculated since \(\Delta P\) is recorded with an electromanometer, and \(r\), the radius of the bubble, is measured through a microscope.

Liquid pressure was first recorded for two minutes while the bubble was of a fixed size (radius = 500\(\mu\)). The bubble was then made to pulsate causing the radius to change from a maximum of 500\(\mu\) to a minimum of 400\(\mu\). The apparent surface tension \(\gamma_{\text{max}}\) and \(\gamma_{\text{min}}\) at maximal and minimal bubble size was calculated, and stability index, \(S\), was obtained by using the formula\(^12\):

\[
S = \frac{2(\gamma_{\text{max}} - \gamma_{\text{min}})}{\gamma_{\text{max}} + \gamma_{\text{min}}}
\]

Lipid analysis

As seen from the Table I, the amount of total lipids in the crude fetal lung fluid, the foam and the residuum was 65 mg%, 140 mg% and 25 mg%, respectively. The content of phospholipids was considerably lower in the residuum than in either foam or the crude lung fluid.

Fig. 1 shows a thin-layer chromatogram of the lipids extracted from the foam and from the residuum. Lecithin, sphingomyelin, phosphatidyletha-

| Table I. Phospholipid Components of the Crude Fetal Lung Fluid, the Foam and the Residuum |
|-----------------------------------------------|----------|----------|----------|
| Total lipids (mg%)                          | Crude fluid | Foam     | Residuum |
|                                               | 65        | 140      | 25       |
| Lipid                                         | mg%       | per cent | mg%       | per cent | mg%       | per cent |
| Lecithin                                      | 17.6      | 27       | 34.0      | 24       | 5.5       | 22       |
| Sphingomyelin                                 | 2.6       | 4        | 7.0       | 5        | 1.0       | 4        |
| Phosphatidyl-ethanolamine                     | 2.6       | 4        | 5.6       | 4        | 0.8       | 3        |
nolamine and lysolecithin were separated, and no difference in their relative proportions was observed in the crude fluid, the foam or the residuum.

**Surface properties**

Surface tension in a nonpulsating bubble in crude lung fluid or foam was 49 dynes/cm after one minute. After two minutes had elapsed, it decreased to 47 dynes/cm in crude fluid and to 44 dynes/cm in the foam. In the residuum it was 71 dynes/cm after one minute and 69 dynes/cm after two minutes. As seen in Fig. 2, the pressure around a pulsating bubble revealed a conspicuous difference
Fig. 2. Pressure tracings demonstrating the rapid formation of a film outlining a bubble in foam, the lesser activity of the crude lung fluid, and the minimal activity of the residuum.

Fig. 3. Stability indices of crude lung fluid, fluid foam, and the residuum.
between water, crude lung fluid, lung fluid foam and the residuum.

In agreement with the formula of Laplace, \( \Delta P = 2\gamma/r \), the pressure gradient, \( \Delta P \), across the surface of a bubble in water was maximal when the bubble radius, \( r \), was minimal. However, in crude lung fluid and particularly in lung fluid foam the \( \Delta P \) was minimal when the bubble radius, \( r \), was minimal. Thus, there was a decrease of apparent surface tension, \( \gamma \), which was relatively greater than that of \( r \). Pressure recording of the residuum initially had a pattern similar to that of water, but after one minute they became irregular indicating that there was still some surface-active material present. The stability index of the foam increased much faster and reached a higher value than that of the crude lung fluid or of the residuum (Fig. 3).

DISCUSSION

A comparison of the analysis of lipids and of the pressure tracings indicates a positive correlation between the concentration of active phospholipids and surface stability. Surface tension in a nonpulsating bubble in fetal lung fluid foam was almost the same as when it was in the crude fluid. However, pulsations demonstrated a conspicuous difference in the surface properties of the two liquids. The pressure amplitude increased much faster in the foam than in the crude fluid; this is reflected in the more rapid increase of the foam’s stability index (Fig. 3). It was as if the foam bubble could almost instantaneously form a film in its surface which resisted any change in bubble size. This, then, explains the apparently very low surface tension at minimal bubble size and the high surface tension at maximal size. During the early adjustment to extrauterine life, the rapid formation of a film with these properties could be important in the prevention of alveolar collapse.

Since analysis of the surface active phospholipids revealed only a quantitative difference in composition, one might postulate that the ability to form an alveolar lining film is directly related to the concentration of the active phospholipids.

This investigation was supported by United Cerebral Palsy Research and Educational Foundation (Dwight D. Eisenhower Fund) and the United States Public Health Service.

References