<table>
<thead>
<tr>
<th>Title</th>
<th>PULMONARY SURFACE TENSION An Approach to the Evaluation of Surface Tension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>Okamoto, Sumitada</td>
</tr>
<tr>
<td>Citation</td>
<td>Acta medica Nagasakiensia. 1970, 14(3-4), p.87-97</td>
</tr>
<tr>
<td>Issue Date</td>
<td>1970-03-25</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10069/15557">http://hdl.handle.net/10069/15557</a></td>
</tr>
</tbody>
</table>

NAOSITE: Nagasaki University’s Academic Output SITE
PULMONARY SURFACE TENSION
—an Approach to the Evaluation of Surface Tension—

Sumitada OKAMOTO *
Second Department of Internal Medicine,
Nagasaki University School of Medicine,
Nagasaki, Japan

Received for publication, February 3, 1970

A study was carried out on the significance of surface tension values of lung washing extracts obtained from dogs consisting of fasted and linoleate-fed groups. The mean maximal surface tension of the latter was lower than that of the former (P < 0.05). The mean minimal surface tension of the latter was higher than the former (not significant). The frequency polygon of the minimal surface tensions was divided into two portions for the linoleate-fed group.

On the other hand, twofold serial dilution of washing extracts was performed and then tension-concentration diagram was drawn with surface tension on its ordinate and phospholipid concentration of the extract on its abscissa.

The tension-concentration diagram showed that the surface tension decreased to some level as the concentration of phospholipid increased, but no further lowering of the tension was observed regardless of increased concentration. Judging from the tension-concentration diagram, it was possible to conceive critical micelle concentration (c.m.c.) in lung washing extracts. There was no significant difference in the pattern between the fasted group and the linoleate-fed group, partly because of the relatively wide range of c.m.c.

Phosphatidyl-choline (Ph–C) was a major component of the washing extract. Ph–C fractions were also isolated from soy-bean lecithin, serum and lung tissues, and the respective values of their minimal surface tensions were 32.1, 28.2 and 28.8 dynes/cm at c.m.c. (0.3 γ of phosphorus). The minimal surface tension of Ph–C isolated from washing extract of the fasted dog attained 3.0 dynes/cm at c.m.c. (15 γ of phosphorus). Ph–C of the linoleate-fed dog demonstrating higher minimal tension value (11.7 dynes/cm) in the crude washing showed the value of 12.6 dynes/cm at the same concentration level (15 γ of phosphorus).

It was concluded from the data that tension-concentration diagram is important for the precise evaluation of surface tension values of lung washing extracts.
INTRODUCTION

Since it was reported by Schrade\textsuperscript{1)} that the lung metabolizes lipids, there have been many reports on lipid metabolism of the lung.

In our department, there were several reports on the lipids of the lung. The lung produced lipoprotein-lipase (LPL) and the administration of fat induced the LPL activities of the lung\textsuperscript{233}. Plasma LPL activities were decreased by ligation of unilateral pulmonary artery\textsuperscript{13}. In trilinolein-fed rats, lipid content especially phospholipids of the lung increased six hours after the administration of trilinolein. Furthermore, it was reported that linoleic acid increased in phospholipid fractions and phosphatidic acid increased in trilinolein-fed rat lung\textsuperscript{56}. Increased phospholipid fractions were observed also in ethyl-linoleate-fed dog lung\textsuperscript{75}. When acetate-C\textsubscript{14} was incubated with tissue slices from dog, the incorporation into total lipid and phospholipid was higher in the lung than in the liver\textsuperscript{56}.

On the other hand, surface tension of the lung was first described by von Neergaard\textsuperscript{99} and has been examined from the viewpoint of pulmonary mechanics\textsuperscript{10}11. Recently indirect measurement of the alveolar surface tension was made possible by several methods using analogous surface of the lung extract\textsuperscript{111213}, but reliable direct assay has not yet been established. Therefore the values of surface tension vary with the respective methods of extraction and surface analogue preparation\textsuperscript{14}.

Surface-active materials of the lung were also studied biochemically, and phospholipids were proved to be potent surface-active materials. Especially, phosphatidyl-choline is the predominant component of the phospholipid extracts\textsuperscript{15}. Thus phospholipids of the lung play an important role in surface activity, though there have been only a few articles on the relationship between surface tension and concentration of washing extract of the lung\textsuperscript{161718}.

This paper describes a possible relationship between surface tension and phospholipid concentration of washing extract of the lung.

MATERIALS AND METHODS

Surface Tension Measurement of Lung Washing Extract

Eighteen mongrel dogs weighing 8–15 kg were fed with normal kennel diet, and divided into two groups. Nine dogs were starved overnight (fasted group). The other nine were also starved overnight, then anesthetized with pentobarbital sodium and fed forcibly with 50 ml of ethyl-linoleate through stomach tubes six hours before the experiment (linoleate-fed group). Both groups were anesthetized just before the experiment and sacrificed by exsanguination. In the linoleate-fed group, the residual ethyl-linoleate in the stomach was
confirmed. Then the entire lung was removed for the study. By means of gravity, the left lower lobe was rinsed five times reciprocally with 100 ml of saline and the recovered washing extract was instilled into the teflon trough of the modified Langmuir-Wilhelmy surface film balance (Acoma Wilhelmy balance) for the measurement of surface tension. The surface was then alternatively compressed to one-fifth of its area, and re-expanded over 2.5 minute cycles, after the first surface aging for 30 minutes. A determination was completed when the two successive surface-area diagrams were accomplished, and recorded on a YEW X-Y recorder. Stability indices were calculated according to the formula provided by Clements et al.:

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

Quantitative Analysis of Lipid in the Washing Extracts and the Lung Tissues

Washing extract in the teflon trough was decanted into a flask for lyophilization after the measurement of surface tension. Extraction of its lipid was then performed according to the method of Folch and Løes. Lung tissue samples each weighing 2.5 g were excised from the left upper lobe, homogenized in glass homogenizers and used for lipid extraction as mentioned above.

Lipid content of the lung tissue and the washing extract was determined by the following methods. The total amount of lipid was measured by gravimetry, phosphorus content by the method of Allen, triglyceride by the method of van-Handel and Silversmit, and total cholesterol by the method of Zak and Henly. Phospholipids were fractionated by thin layer chromatography and phosphorus content of their subfractions was determined by the micro-quantitative method of Nojima. Fatty acid residue of them was methyl-esterified by the method of Stoffel, and then determined by gas-liquid chromatography (Shimadzu Gas Chromatograph GC-IB).

Tension-Concentration Diagram of Lung Washing Extract

Six mongrel dogs weighing 10–12 kg were divided into two groups of three fasted dogs and three linoleate-fed ones. The treatment of them was performed by the same method as has previously been mentioned. After sacrificing by exsanguination, the entire lung was removed for the study and blood in the pulmonary vessels was washed out with approximately 200 ml of saline. Then both lower lobes were washed twice with 200 ml of saline. Fifty milli-liters of the recovered extract was instilled into the trough for the measurement of surface tension, and 50 ml of extract separated from the rest was decanted into a beaker for dilution. Then twofold serial dilution of the extract
was performed up to 32768 x in its dilution level. During dilution, continuous agitation of the diluents was performed by a magnetic stirrer to preserve their homogeneity, and the process of dilution was accomplished within 20 minutes to prevent the diluents from aging. Surface tensions of them were also determined by the surface film balance. Tension-concentration diagram was drawn with surface tension on its ordinate and cube root of phospholipid concentration of the diluents on its abscissa for the convenience of analysis. Mean surface tension values of three samples versus phospholipid concentration of the diluents were plotted on the diagram. Phospholipid concentration was calculated from the dilution ratio.

**Tension-Concentration Diagram of Phosphatidyl-Choline Samples**

A dog was selected at random each from the fasted group, the linoleate-fed group with high minimal surface tension (11.7 dynes/cm) and the linoleate-fed group with low minimal surface tension (3.0 dynes/cm), and its respective phosphatidyl-choline sample was prepared.

Phosphatidyl-choline fractions were isolated from washing extract, lung tissue, serum and soy-bean lecithin by thin layer chromatography. Phosphorus determination was done by the method of ALLEN. Each phosphatidyl-choline fraction was made up in 100 μ/ml and 1 μ/ml solution in respect of phosphorus concentration in chloroform, and an aliquot of solution was gently layered through micro-syringe on the surface of saline in the trough. Surface tension was determined after aging. Then, additional aliquots were layered serially with the determination of surface tensions. Tension-concentration diagram was also drawn.

**RESULTS**

**Surface Tension of the Lung Washing Extracts of the Fasted Dogs and Linoleate-fed Ones**

The mean maximal and minimal surface tensions of the washing extracts were 37.7±3.8 and 4.9±2.4 dynes/cm in the fasted group, and 33.9±2.0 and 7.6±4.6 dynes/cm in the linoleate-fed group respectively. The stability index was 1.56±0.13 in the fasted group, and 1.30±0.40 in the linoleate-fed group. The mean maximal surface tension of the latter was lower than that of the former (P<0.05). The mean minimal surface tension of the latter was higher than that of the former (not significant). The stability index of the latter was lower than that of the former (not significant).

The frequency polygon of surface tensions and stability indices demonstrated that the distribution of maximal and minimal surface
tensions of the fasted group and maximal surface tensions of the linoleate-fed group formed only one peak respectively, but that the same of minimal surface tensions of the linoleate-fed group formed two peaks (Fig. 1). The polygon of the stability indices also demonstrated only one peak for the fasted group but two peaks for the linoleate-fed group (Fig. 2).

Quantitative Analysis of Lipid of the Washing Extracts and the Lung Tissues

The amount of recovered washing extract was 70±4 ml in the

Table 1 Lipid Fraction of Lung Tissue and Washing Extract (%) (8 dogs)

<table>
<thead>
<tr>
<th>Lipid Component</th>
<th>Lung Tissue</th>
<th>Washing Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Linoleate-fed</td>
<td>Normal Linoleate-fed</td>
</tr>
<tr>
<td>Total Amounts</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>33</td>
<td>29</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>61</td>
<td>67</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Lysolecithin</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Undetermined spot</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 1 Lipid Fraction of Lung Tissue and Washing Extract (%) (8 dogs)
fasted group and 68±8 ml in the linoleate-fed. The difference was not significant.

Phosphorus concentration of the washing extracts was 210±120 μg/50 ml in the fasted group, and 64±29 μg/50 ml in the linoleate-fed group. (P<0.01)

Table 1 shows the relative lipid content of the washing extracts and the tissues. The total lipid content was slided in scale to 100%. Triglyceride occupied 10% of total lipid in the fasted group and 17% in the linoleate-fed group.

As to the lipid content of the lung tissues, there was no significant difference in the amount of total lipid, phospholipid, total cholesterol and triglyceride between the two groups.

*Tension-Concentration Diagram of Lung Washing Extract (Fig. 3)*

In the range of phospholipid concentration less than $1.12 \times 10^8$ g. mol./L., the maximal and minimal surface tensions revealed the same value (73.8 dynes/cm). As the concentration increased, the tension first decreased rapidly to some level and thereafter decreased gradually. Their respective values attained 33.3 dynes/cm at the concentration of $3.68 \times 10^{-4}$ g. mol./L., and 3.0 dynes/cm at the concentration of approximately $9.22 \times 10^5$ g. mol./L. Especially the minimal surface tension maintained a constant value in the range of concentration more than $9.22 \times 10^5$ g. mol./L. in like manner that other highly surface-active agents demonstrated in the range of their critical micelle concentration (c.m.c.).

There was no significant difference in tension-concentration diagram between the fasted group and the linoleate-fed group.
Tension-Concentration Diagram of Phosphatidyl-Choline Samples

Fig. 4 shows the tension-concentration diagram of phosphatidyl-choline fractions of washing extract, lung tissue and serum in the selected fasted dog. As the concentration increased, the minimal surface tension of lung washing phosphatidyl-choline decreased step-wise and reached 3.0 dynes/cm at the phosphorus concentration of approximately 15γ. The minimal surface tensions of lung tissue phosphatidyl-choline and serum phosphatidyl-choline also decreased with the increase of phosphorus concentration and attained 25.8 and 27.6 dynes/cm respectively, in the range of phosphorus concentration more than 0.3γ.

Fig. 5 shows the tension-concentration diagram of the phosphatidyl-choline fraction isolated from soy-bean lecithin and from the selected linoleate-fed dogs.

Washing phosphatidyl-choline of the linoleate-fed dog with low minimal surface tension was practically of the same pattern as that
of the fasted dog. On the other hand, the same of the linoleate-fed dog with high minimal surface tension showed more gradual decrease of tension than that of the fasted one. Phosphatidyl-choline samples of soy-bean lecithin attained 30.0 dynes/cm of minimal surface tension at the phosphorus concentration level more than 0.3γ.

The phosphatidyl-choline fraction of soy-bean lecithin and that of the linoleate-fed dog with high minimal surface tension, demonstrated the same maximal surface tension values of 42.6 dynes/cm at the phosphorus concentration of 40γ. Those values were lower than the value of 56.4 dynes/cm in the linoleate-fed dog with lower minimal surface tension.

**Fatty Acid Residues of Phosphatidyl-Choline Samples**

Table 2 shows the relative content of fatty acid residues of phosphatidyl-choline samples. It was 13.5% for 16:0, and 66.0% for 18:2 in soy-bean lecithin, 16.6% for 16:0 and 14.8% for 18:2 in serum, 32.6% for 16:0 and 14.3% for 18:2 in lung tissue from the fasted dog, and 55.7% for 16:0 and 2.5% for 18:2 in washing extract from the fasted dog.

It was 37.6% for 16:0, and 8.4% for 18:2 in washing extract from the linoleate-fed dog with low minimal surface tension. And it was 41.2% for 16:0, and 2.8% for 18:2 in washing extract from the linoleate-fed dog with high minimal surface tension.

There were significant differences in the relative content of fatty acid residues among soy-bean lecithin, serum, lung tissue and washing extract. It was not determined whether or not the difference in the fatty acid composition of the washing phosphatidyl-choline was significant among the dogs with high and low minimal surface tensions.

<table>
<thead>
<tr>
<th>Table 2 Fatty Acid Residues of Phosphatidyl-Choline Fractions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Lung Washing Extract</td>
</tr>
<tr>
<td>Fasted</td>
</tr>
<tr>
<td>Linoleate-fed (low γ-min)</td>
</tr>
<tr>
<td>Linoleate-fed (high γ-min)</td>
</tr>
<tr>
<td>Lung Tissue</td>
</tr>
<tr>
<td>Fasted</td>
</tr>
<tr>
<td>Linoleate-fed (low γ-min)</td>
</tr>
<tr>
<td>Serum</td>
</tr>
<tr>
<td>Soy-bean lecithin</td>
</tr>
</tbody>
</table>
DISCUSSION

Mean minimal surface tension of the lung washing extracts increased and mean phosphorus concentration decreased in the linoleate-fed group as compared with the fasted group. However, it was questionable whether or not the difference of the mean minimal surface tension was significant, because surface tension depends also on the amount of surfactants. The difference of phosphorus concentration might result from insufficient recovery of phospholipids from the alveolar lining layer of the linoleate-fed dogs.

The frequency polygon of minimal surface tension and stability index was divided into two portions. Accordingly, it is a question to attempt to determine the significant difference among those values. For the evaluation of those values, it is necessary to adjust them in consideration of the amounts of the recovered surfactants.

There have been several reports which suggest the quantitative relationship between surface tension of body fluid and its concentration. It was reported that lipid phosphorus contents of mincing extract correlated inversely with minimal surface tension in the lung tissues of mongrel dogs after unilateral pulmonary ligation\textsuperscript{16}.

Adam\textsuperscript{17} reported that diluted fetal lamb tracheal fluid caused an increase in surface tension. Brown\textsuperscript{18} investigated on the effect of dilution of rabbit lung washing and suggested that both the minimal and maximal tension rose rapidly at some level of dilution and that the surface area calculated from tension-area diagram was broken.

In this study, extract was serially diluted to the level of 32768 x for more precise tension-concentration diagram. The curve of minimal surface tension was approximately fitted to exponential relationship. By the adaptation of Gibb's adsorption equation\textsuperscript{23} to the tension-concentration curve of lung washing extract, it was speculated that logarithmic increase of concentration to some level causes constant adsorption of lung extracts and thereafter induces no further adsorption on the surface.

From this point of view, the tension-concentration diagram of lung washing extracts was simulated to that of general surface-active agent, and hence critical micelle concentration (c.m.c.) of lung washing extracts was supposed to be determinable. In this experiment, however, c.m.c. was determined only as a probable range of concentration which could be affected by many factors. Those factors were speculated to be inhomogeneity of extracts in dilution, recovery of phospholipids which effectively participated in film-formation, and inhibitors such as triglyceride\textsuperscript{29}.

Crude lung washing extracts contain many components affecting surface tension, and phosphatidyl-choline is generally accepted as a major component of surface-active phospholipids of lung extracts.
Hence phosphatidyl-choline was isolated from lung washing extracts of the fasted dog, lung washing extracts of the linoleate-fed dog, lung tissue, serum and soy-bean lecithin. These phosphatidyl-choline samples were used for tension-concentration diagram.

Tension-concentration diagram of the phosphatidyl-choline samples demonstrated significant differences among lung washing extract, lung tissue, serum and soy-bean lecithin. The respective minimal surface tensions of soy-bean lecithin, serum and lung tissue were 32.1, 28.2, and 28.8 dynes/cm at c.m.c. of 0.3\(\gamma\) of phosphorus, and lung washing extracts attained 3.0 dynes/cm at c.m.c. of 15\(\gamma\) of phosphorus. As concentration increased, the minimal surface tension of lung washing extracts first decreased sharply in the concentration range of approximately 0.02 to 0.3\(\gamma\) of phosphorus, then maintained an approximately constant value in the range of 0.3 to 0.6\(\gamma\) of phosphorus, decreased gradually in the range of 0.6 to 15\(\gamma\) of phosphorus, and maintained again a constant value in the range more than 15\(\gamma\) of phosphorus. The minimal surface tensions of soy-bean lecithin, serum, and lung tissue also decreased sharply in the concentration range below 0.3\(\gamma\) of phosphorus, but the tension values remained nearly constant in the range more than 0.3\(\gamma\) of phosphorus.

In the composition of fatty acid residues, phosphatidyl-choline of the lung washing extract contained a relatively large amount of saturated fatty acids, and phosphatidyl-choline of soy-bean lecithin, serum and lung tissue contained a relatively large amount of unsaturated fatty acids.

It was concluded from the tension-concentration diagram that the difference in the patterns among the four samples was not due to the difference in quantity of those surfactants, but due to the difference in their qualitative properties.

In the linoleate-fed dogs, there were two groups whose washings each attained relatively high and relatively low minimal surface tensions. In the dog with high minimal surface tension, the minimal surface tension of its phosphatidyl-choline sample decreased with the increase of phosphorus concentration, and attained 12.6 dynes/cm at the concentration of 15\(\gamma\) of phosphorus. This pattern differed from that of the fasted dog, but it was not concluded that the difference of surface tensions between the two groups was due to that of fatty acid compositions.

A more gradually decreasing minimal surface tension curve, lower maximal surface tension, and instability of hysteresis of tension-area diagram were demonstrated by the phosphatidyl-choline sample of the dog with high minimal surface tension.

It is generally accepted that the balance method cannot be quantitative. Indeed, it was proved in the present study as well that the balance method cannot be quantitative at the concentration more than
c.m.c. Therefore, it is necessary to determine by tension-concentration diagram whether the concentration of the surface-active material is in the range more than c.m.c. Although application of the tension-concentration diagram to crude washing extracts is difficult because of the factors mentioned previously, the tension-concentration diagram is useful to analyse surface tensions of phosphatidyl-choline from the washing extracts and to evaluate precisely surface tension values of washing extracts.

ACKNOWLEDGEMENT

The author is grateful to Professor SHIRO OSAJIMA, YASUTAKA KATOH M.D. and KIYOSHI KANZAKI M. D. for their continuous helpful advice and encouragement.

REFERENCES

4) Kikitsu, Y. (personal observations and unpublished experiments)
8) Kimura, N., Mori, I., Hanuno, T. (personal observations and unpublished experiments)
26) Nojima, S. : Metabolism and Disease, 2 : 1023. 1965. (Japanese)