Pulmonary Surface Tension and Phospholipid Metabolism in Diplococcal and Mycoplasmal Pneumonia

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In attempt to evaluate the mechanism, by which the tendency of atelectasis was produced in diplococcal and mycoplasmal pneumonia, this study was done.

Hamsters were used as materials. The result showed the low surface activity in diplococcal and mycoplasmal pneumonia. Furthermore, the cause of the low surface activity was investigated especially from the point of the quantitative and qualitative alteration of phospholipids which was a major component of pulmonary surfactants. The results suggested that the decrease of saturated fatty acids and the increase of unsaturated fatty acids of the lung extracts was a factor, by which the low surface activity was induced in both pneumonic groups.

On the other hand, the lung extracts contained erythrocytes in the pneumonic group. Hence, in the pneumonic group the low surface activity may result from the contamination due to the exudation of intravascular component such as serum. Fatty acid composition of lecithin of the lung extracts in pneumonic group was not similar to that of serum lecithin.

In consequence, it was suggested that the qualitative alteration of lecithin of the lung extracts might be a factor of the low pulmonary surface activity.

INTRODUCTION

Since it was reported Schrade1) that lipid contents of the lung showed a remarkable increase, especially phospholipid contents, in the
rats orally administered olive oil, many studies have been reported on the lipid metabolism of the lung.

On the other hand, the lung has a external respiratory function. The relation between lipids and the external respiratory function had been investigated but remained unsolved.

The fact that the fat administered orally is sent to the lung through the thoracic duct as chylomicron after the absorption by intestine, i.e., this physio-anatomical characteristics suggests some special relation between the external respiratory function and lipid metabolism of the lung.

On the other hand, the pulmonary surface tension was first described by Von Neergaard\(^2\) and Pattle\(^3\) described in 1955 a method for studying the surface properties of the lung by the determination of the stability of lung babbles. However, most of recent studies\(^4\)\(^5\) of pulmonary surface properties have been made by the measurement of the surface tension of the lung extracts on a surface balance.

The latter method has an advantage in studying the relation of surface tension to the changes of surface area.

Recent studies\(^6\)\(^7\)\(^8\) also have revealed that the surface active material is lipoprotein in nature and its phospholipids, especially lecithin, was proved to be a potent surface active material.

Furthermore, surface properties in some pathological conditions such as pulmonary edema\(^9\), atelectasis of the lung\(^10\)\(^11\) and pneumonia\(^12\) has been studied.

Sutnick\(^12\) elucidated the low surface properties of the pneumonic lung of the patients, but there was no report in which the cause of the low surface properties of the pneumonic lung and the correlation between this low surface properties and phospholipid metabolism were investigated.

This paper describes the possible relationship of pulmonary surface tension and phospholipid metabolism of pulmonary surfactants in diplococcal and mycoplasmal pneumonia.

**MATERIALS**

I) Five normal adult hamsters weighing 100—120 g were fed with normal rat diet and they were used as normal adult group for the control.

II) One ml of triptosoy case broth culture medium containing diplococcus pneumoniae \((10^8 /\text{ml})\) was administered to normal adult hamster by rhinenchysis. Ten infected hamsters were prepared as the diplococcal pneumonic group and were used at 48 hours after the infection. On the other hand, three weeks old hamster is the most favourable to
the infection of mycoplasmal pneumonia, so young hamsters of three weeks old were used as materials of mycoplasmal pneumonia. As the body weight of two young hamsters is nearly equal to that of an adult hamster, two young hamsters were used as one young material.

III) Ten normal young hamsters weighing 55–50 g, i.e., five normal young materials were used as normal young group for the control.

IV) Twelve normal young hamsters weighing 50–55 g, i.e., six normal young materials were infected with mycoplasma pneumoniae by following method. Hamsters were kept in a container such as a desiccator and were exposed for 30 minutes to the moisture of FH-strain suspension broth containing $2.6 \times 10^6$ colony forming unit per ml. The infected hamsters were used as mycoplasmal pneumonic group at 14 days after the infection. These materials were revealed to contain from 4 to 8 times of CF antibody.

**METHODS**

(A) Surface tension of the mincing extracts.

These four group mentioned above were used as materials. The both lungs of each hamster in each group were removed after 24 hours starvation. The both removed lungs were perfused by 10 ml of saline, and then 2 ml of saline was inserted for the sufficient inflation through the main bronchus. It was kept in the inflated condition for 15 minutes to get more sufficient alveolar surfactants and then these inflated lungs were minced by scissors. Thirty five ml of saline were added to the minced lung and this solution was stirred by magnetic stirrer for 15 minutes. This sample was strained through a fourfold thickness of guaze and was divided into the crude mincing extracts and lung tissue. Furthermore, this crude mincing extracts was divided into the supernate and precipitate by the centrifugation (1500 r.p.m., 15 minutes). For the measurement of the pulmonary surface tension this supernate was instilled into the teflon trough of the modified Langmuir-Wilhelmy surface film balance (Acoma Wilhelmy Balance). The surface then alternatively was compressed to one-fifth of its area and re-expanded over 2.5 minutes cycles after the first surface aging for 2 hours. A determination was completed when two successive surface-area diagram were accomplished and recorded on a X-Y recorder. Stability index of each sample was calculated according to the formula provided by Clements et al.

$$S.I. = \frac{2(\gamma_{\text{max}} - \gamma_{\text{min}})}{(\gamma_{\text{max}} + \gamma_{\text{min}})}$$

(B) Quantitative analysis of phospholipid in the mincing extracts and lung tissues.
The supernate was decanted into the lyophilyzing flask after the measurement of surface tension. After lyophilization, lipid extraction from the supernate was then performed according to the method of Folch and LEES\(^{14}\). On the other hand, the lung tissues were homogenized in glass-homogenizer and its lipid extraction was performed by the same method. Phospholipid contents of the lung tissues and the supernate was determined by the method of ALLEN\(^{15}\). Furthermore, phospholipid wasfractionated by thin-layer chromatography\(^{16}\) and phospholipid contents of its subfractions was determined by the micro-quantitative method of NOJIMA\(^{17}\).

(C) Gas liquid chromatographic analysis of lecithin.

For qualitative analysis of lecithin which was identified in the preliminary experiment as a major component of phospholipid of the lung tissue and the supernate, six normal adult hamsters, six hamsters infected with diplococcus pneumoniae, ten normal young hamsters (five normal young materials) and twelve young hamsters infected with mycoplasma pneumoniae (six materials) were prepared. Phospholipid extraction from the lung tissues and the supernate in each group was performed by the same method. Fatty acid residue of lecithin of the lung tissues and the supernate was methyl-esterified by the method of STOFFEL\(^{18}\) and then determined by gas liquid chromatography (SHIMAZU Gas Chromatograph, GC-IB). Fatty acid composition of serum lecithin was also determined by the same method.

(D) Tension-concentration diagram of fatty acids.

Miscellaneous fatty acids such as myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and arachidic (20:0) were prepared for the investigation of the correlation between the surface tension and fatty acids. Each fatty acid was dissolved in the solvent of chloroform: methanol: n-hexane (1:1:3) according to the method of SHAN and SCHULMAN\(^{19}\). Each fatty acid solution was gently layered through microsyringe on the surface of 35 ml of saline in the trough, and the relation between minimal surface tension and the concentration of each fatty acid was obtained as tension-concentration diagram.

(E) Cell count in the precipitates.

As an index of influence of intravascular component on the surface activity, erythrocytes per 0.1 mm\(^3\) were counted using TURK’s calculator after adjusting to be five ml in volume by addition of saline to the precipitates.
RESULTS

(1) Fig. 1 showed the maximum and minimal surface tension of the supernate in each group. The minimal surface tension in the diplococcal pneumonic group was higher than that in the normal adult group (significant). The minimal surface tension in the mycoplasmal pneumonic group was also higher than that in the normal young group (significant). Fig. 2 showed stability index of the supernate in each group. Stability index in the diplococcal pneumonic group was lower than that in the normal adult group, and stability index in the mycoplasmal pneumonic group was lower than that in the normal

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**Fig. 1. Surface Tension of the Supernate in each Group**

<table>
<thead>
<tr>
<th>N.A.G.</th>
<th>D.P.G.</th>
<th>N.Y.G.</th>
<th>M.P.G.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>40</td>
<td>50</td>
<td>60</td>
<td>70</td>
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</table>

N.A.G.: Normal Adult Group  
D.P.G.: Diplococcal Pneumonic Group  
N.Y.G.: Normal Young Group  
M.P.G.: Mycoplasmal Pneumonic Group

**Fig. 2. Stability Index of the Supernate in each Group**

<table>
<thead>
<tr>
<th>N.A.G.</th>
<th>D.P.G.</th>
<th>N.Y.G.</th>
<th>M.P.G.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

N.A.G.: Normal Adult Group  
D.P.G.: Diplococcal Pneumonic Group  
N.Y.G.: Normal Young Group  
M.P.G.: Mycoplasmal Pneumonic Group

**Fig. 3. Total Lipid-Phosphorus of the Lung Tissue (1 g)**

<table>
<thead>
<tr>
<th>N.A.G.</th>
<th>D.P.G.</th>
<th>N.Y.G.</th>
<th>M.P.G.</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>200</td>
<td>300</td>
<td>400</td>
</tr>
<tr>
<td>500</td>
<td>600</td>
<td>700</td>
<td>800</td>
</tr>
</tbody>
</table>

N.A.G.: Normal Adult Group  
D.P.G.: Diplococcal Pneumonic Group  
N.Y.G.: Normal Young Group  
M.P.G.: Mycoplasmal Pneumonic Group
young group (significant).

(2) For the investigation of the correlation between the lower surface properties and phospholipid of both infection group, total lipid-phosphorus of the lung tissues and the supernate was measured. Fig. 3 showed total lipid-phosphorus of the lung tissues (1 g). The significant alteration in both infection groups was not proved as compared with both normal control groups. Fig. 4 showed total lipid-phosphorus of the supernate of the mincing extracts from the

Fig. 4. Total Lipid-Phosphorus of the Supernate extracted from the Lung Tissue (1g)

N.A.G.: Normal Adult Group
D.P.G.: Diplococcal Pneumonic Group
N.Y.G.: Normal Young Group
M.P.G.: Mycoplasmal Pneumonic Group

Fig. 5. Lecithin-Phosphorus/Total Lipid-Phosphorus and Minimal Surface Tension of the Supernate in each Group

○: Normal Adult Group
●: Diplococcal Pneumonic Group
△: Normal Young Group
▲: Mycoplasmal Pneumonic Group
lung (1 g). Total lipid-phosphorus of the supernate in each infection group was more than that in each normal control group in spite of the low surface activity in each infection group.

(3) On the other hand, PATTLE or FINLEY pointed out that a predominant component of the lung extracts was lecithin. Hence, phosphorus of lecithin was determined. Fig. 5 showed the correlation between percentage of lecithin-phosphorus/total lipid-phosphorus and minimal surface tension of the supernate in each group. Fig. 6 showed the correlation between percentage of lecithin-phosphorus/total lipid-phosphorus and stability index of the supernate in each group. But there was no certain correlation between lecithin-phosphorus and the surface activity of the supernate in each infection group and each normal control group.

(4) The low surface properties in both pneumonic groups were investigated in point of quality of lecithin of the supernate and the lung tissues, so fatty acid composition of lecithin of the supernate and the lung tissues was determined (Table 1). Furthermore, fatty acid composition of serum lecithin was determined for the investigation of the influence of the intravascular component on the surface activity.

Fig. 6. Lecithin-Phosphorus/Total Lipid-Phosphorus and Stability Index of the Supernate in each Group

The graph shows the correlation between Lecithin-Phosphorus/Total Lipid-Phosphorus (%) and Stability Index. The data points are differentiated by the type of group:
- ○: Normal Adult Group
- ●: Diplococcal Pneumonic Group
- △: Normal Young Group
- ▲: Mycoplasmal Pneumonic Group
(Table 1). Fig. 7 showed fatty acid composition of lecithin of the supernate in the normal adult group, the diplococcal pneumonic group and of seurm lecithin. Fig. 8 shows fatty acid composition of lecithin of the lung tissues in these two groups and of serum lecithin. Fig. 7

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>12:0</th>
<th>14:0</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:4</th>
</tr>
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<tbody>
<tr>
<td>Supernate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N.A.G.</td>
<td>1.7±0.2</td>
<td>1.2±0.4</td>
<td>61.1±1.6</td>
<td>9.6±0.9</td>
<td>3.6±0.8</td>
<td>13.9±1.5</td>
<td>4.8±0.8</td>
<td>1.9±0.3</td>
</tr>
<tr>
<td>D.P.G.</td>
<td>1.5±0.2</td>
<td>0.9±0.2</td>
<td>54.9±0.6</td>
<td>11.1±0.8</td>
<td>3.2±0.5</td>
<td>16.1±0.9</td>
<td>7.0±0.3</td>
<td>0.3±0.2</td>
</tr>
<tr>
<td>N.Y.G.</td>
<td>1.3±0.2</td>
<td>0.1±0.2</td>
<td>61.4±0.3</td>
<td>7.6±0.6</td>
<td>3.3±0.1</td>
<td>15.7±0.5</td>
<td>5.9±0.6</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>M.P.G.</td>
<td>1.1±0.2</td>
<td>0.8±0.2</td>
<td>58.6±0.6</td>
<td>9.7±0.7</td>
<td>2.3±0.5</td>
<td>17.4±0.4</td>
<td>6.2±0.4</td>
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<td>Lung</td>
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</tr>
<tr>
<td>N.A.G.</td>
<td>1.2±0.3</td>
<td>1.0±0.3</td>
<td>48.9±1.0</td>
<td>6.7±0.7</td>
<td>8.4±0.3</td>
<td>16.5±0.7</td>
<td>10.4±0.6</td>
<td>0.3±0.4</td>
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<tr>
<td>D.P.G.</td>
<td>0.8±0.2</td>
<td>0.6±0.2</td>
<td>45.1±0.8</td>
<td>8.8±1.3</td>
<td>6.2±1.2</td>
<td>19.0±1.0</td>
<td>11.8±0.7</td>
<td>5.3±0.4</td>
</tr>
<tr>
<td>Tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N.Y.G.</td>
<td>1.0±0.1</td>
<td>0.8±0.1</td>
<td>46.6±0.4</td>
<td>5.7±0.2</td>
<td>7.6±0.4</td>
<td>19.3±0.3</td>
<td>10.6±0.4</td>
<td>6.3±0.1</td>
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<tr>
<td>M.P.G.</td>
<td>0.7±0.2</td>
<td>0.5±0.1</td>
<td>44.7±1.5</td>
<td>6.5±0.2</td>
<td>6.8±0.6</td>
<td>20.5±0.5</td>
<td>11.7±0.4</td>
<td>6.8±0.3</td>
</tr>
<tr>
<td>Serum Lecithin</td>
<td>0.6±0.2</td>
<td>1.2±0.2</td>
<td>34.2±1.6</td>
<td>2.4±0.3</td>
<td>13.9±0.6</td>
<td>10.8±0.6</td>
<td>24.7±1.0</td>
<td>1.46±0.2</td>
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</tbody>
</table>

N.A.G.: Normal Adult Group
N.Y.G.: Normal Young Group
D.P.G.: Diplococcal Pneumonic Group
M.P.G.: Mycoplasmal Pneumonic Group

Fig. 7. Fatty Acid Composition of Lecithin of the Supernate in the Normal Adult Group, the Diplococcal Pneumonic Group and Serum Lecithin

- : Normal Adult Group
- : Diplococcal Pneumonic Group
- : Serum Lecithin
and Fig. 8 showed that all saturated fatty acids of lecithin of the supernate and the lung tissues in the diplococcal pneumonic group decreased in comparison with those in the normal adult group, and that all

Fig. 8. Fatty Acid Composition of Lecithin of the Lung Tissue in the Normal Adult Group, the Diplococcal Pneumonic Group and Serum Lecithin

![Bar chart showing fatty acid composition.]

Fig. 9. Fatty Acid Composition of Lecithin of the Supernate in the Normal Young Group, the Mycoplasmal Pneumonic Group and Serum Lecithin

![Bar chart showing fatty acid composition.]

unsaturated fatty acids of lecithin of them in the diplococcal pneumo-
nic group increased in comparison with those in the normal adult
group. But the alteration of each fatty acid in the diplococcal
pneumonic group had not necessarily a tendency to near the fatty acid
composition of serum lecithin. Fig. 9 showed fatty acid composition
of lecithin of the supernate in the normal young group, the mycoplas-
mal pneumonic group and of serum lecithin. Fig. 10 showed fatty
acid composition of the lung tissues in the normal young group, the
mycoplasmal pneumonic group and of serum lecithin. Fig. 9 and Fig.
10 showed that all saturated fatty acids of lecithin of the supernate
and the lung tissues in the mycoplasmal pneumonic group decreased
in comparison with those in the normal young group, and all unsatu-
rated fatty acids of lecithin of them in the mycoplasmal pneumonic
group increased in comparison with those in the normal young group.
But fatty acid composition in the mycoplasmal pneumonic group had
not necessarily a tendency to near that of serum lecithin. Fig. 11
showed the correlation between percentage of palmitic acid/total fatty
acid of lecithin and minimal surface tension of the supernate in each
group, i.e., each pneumonic group showed the low proportion of
palmitic acid/total fatty acid and higher minimal surface tension in
comparison with those in each normal group. Fig. 12 showed the
correlation between percentage of palmitic acid/total fatty acids of
lecithin and stability index of the supernate in each group. That is,
each pneumonic group showed the low percentage of palmitic acid/
total fatty acids and the low stability index of the supernate in com-
parison with those in each normal control group.

Fig. 10. Fatty Acid Composition of Lecithin of the Lung Tissue in the Normal
Young Group, the Mycoplasmal Pneumonic Group and Serum Lecithin

- Normal Young Group
- Mycoplasmal Pneumonic Group
- Serum Lecithin
Fig. 11. Palmitic Acid/Total Fatty Acid of Lecithin and Minimal Surface Tension of the Supernate in each Group

Fig. 12. Palmitic Acid/Total Fatty Acid of Lecithin and Stability Index of the Supernate in each Group
Fig. 13. Total Saturated Fatty Acid (T.S.F.A.)/Total Unsaturated Fatty Acid (T.U.F.A.) of Lecithin and Minimal Surface Tension of the Supernate in each Group.

Fig. 14. Total Saturated Fatty Acid (T.S.F.A.)/Total Unsaturated Fatty Acid (T.U.F.A.) of Lecithin and Stability Index of the Supernate in each Group.
Fig. 15. Tension-Concentration Diagram of Fatty Acids

1: Linoleic Acid (18:2)
2: Oleic Acid (18:1)
3: Myristic Acid (14:0)
4: Palmitic Acid (16:0)
5: Stearic Acid (18:0)
6: Arachidic Acid (20:0)

Fig. 16. Correlation between Minimal Surface Tension of the Supernate and Erythrocytes Count in the Precipitate

- O: Normal Adult Group
- ●: Diphtesical Pneumonic Group
- △: Normal Young Group
- ▲: Mycoplasmal Pneumonic Group
Furthermore, a proportion of total saturated fatty acids/total unsaturated fatty acids of lecithin of the supernate in each group was:

the normal adult group .................. 2.25±0.11
the diplococcal pneumatic group .......... 1.70±0.17
the normal young group .................. 2.18±0.05
the mycoplasmal pneumatic group ........ 1.80±0.07

Fig. 13 and Fig. 14 showed the correlation between a proportion of total saturated fatty acids/total unsaturated fatty acids of lecithin and minimal surface tension or stability index of the supernate in each group.

Fig. 17. Relative Ratio of each Fatty Acid of Lecithin of the Supernate in the Normal Adult Group, the Diplococcal Pneumatic Group and Serum Lecithin
Each pneumonic group showed a low proportion of total saturated fatty acids/total unsaturated fatty acids of lecithin and low surface activity of the supernate in comparison with those in each normal control group.

(5) Hence, for the investigation of the correlation between various kinds of fatty acids and the surface properties, tension-concentration diagram of miscellaneous fatty acids such as myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and arachidic acid was drawn (Fig. 15).

**Fig. 18. Relative Ratio of each Fatty Acid of Lecithin of the Supernate in the Normal Young Group, the Mycoplasmal Pneumonic Group and Serum Lecithin**
The minimal surface tension decreased stepwise with the increase of fatty acid concentration, and the more carbon number became and the higher saturation fatty acid had, the higher surface activity became.

(6) On the other hand, the low surface activity in each pneumonic group may result from contamination due to intravascular components such as serum by the infection of the lung. Therefore, for the index of the influence of contaminations, erythrocytes /0.1 mm$^3$ in the precipitate of the mincing extracts was counted.

Fig. 16 showed the correlation between minimal surface tension of the supernate and erythrocytes count in the precipitate in each group. Erythrocytes count in both normal control groups were less than 100/0.1 mm$^3$ and both normal control groups showed low minimal surface tension. Erythrocytes count in both pneumonic groups were more than 100/0.1 mm$^3$, particularly they were more than 250/0.1 mm$^3$ and less 1230/0.1 mm$^3$ in diplococcal pneumatic group, and both pneumonic groups showed high minimal surface tension. Hence, the contamination due to the intravascular components by infection of the lung might be a factor of low surfactant activity.

(7) Fig. 17 showed the ratio of each fatty acid of lecithin of the supernate in the diplococcal pneumatic group and of serum lecithin when each fatty acid of lecithin of the supernate in the normal adult group was supposed to be one hundred. Fig. 18 showed the ratio of each fatty acid of the supernate in the mycoplasmal pneumatic group and of serum lecithin when each fatty acid of lecithin of the supernate in the normal young group was supposed to be one hundred.

In both pneumonic groups, some of fatty acids of lecithin of the supernate such as 12:0, 16:0, 18:2 and 20:4 near those of serum lecithin. But some fatty acids such as 14:0, 16:1, 18:0 and 18:1 were not similar to those of serum lecithin.

Therefore, the low surface activities in both pneumonic groups were not explained only by the contamination due to the intravascular components.

DISCUSSION

It has been recognized that the patients with pneumonia often exhibited greater abnormality of respiratory function than that expected from the involvement based on roentogenographic changes and clinical evaluation. This phenomenon was evaluated by CoLP$^{21}$. This discrepancy suggests some additional factors to explain this disability. GAIRDNER$^{22}$ pointed out that atelectasis in pneumonia was due to the obstruction from bronchial secretion. But six cases reported by BURBANK$^{23}$ presented the absent breathsound and he attempted to explain
atelectasis on the basis of the force of the surface tension. Furthermore, SUTNICK and SOLOFF\(^\text{24}\) observed that the surface activity was diminished in the lung extracts of the infected lobes. They\(^\text{12}\) measured the pulmonary surface tension of autopsy lung of four patients with pneumonia, i.e., twenty two of twenty three pneumonic specimens were low in surface activity and eleven of nineteen grossly uninfected lobes were also abnormal and four had the evidence of atelectasis.

To explain the low surface activity in pneumonia, SUTNICK\(^\text{12}\) postulated the presence of a surfactant antagonist in the pneumonic lung. TIERNEY and JOHNSON\(^\text{25}\) demonstrated that the surface activity was diminished by heating. Therefore, the influence of fever by infection may be considered as a factor.

But detailed mechanism of the low surface activity in pneumonic lung has been remained unsolved. On the other hand, the pulmonary surfactants is thought to be phspholipid,\(^\text{26}\) particularly dipalmitoyl-lecithin, but the correlation between the low surface activity and phospholipid in pneumonia was not also established.

In this study, not only diplococcal pneumonic specimens but also mycoplasmal pneumonic specimens were low in the surface activity. These results may support the presence of atelectasis of the lung due to mycoplasmal pneumonia described by GEORGE\(^\text{27}\). On the other hand, in spite of the low surface activity of the lung extracts in both pneumonic groups, total phospholipid of the lung extracts increased more than that of normal group. This increase of phospholipid in each pneumonic group may result from exudation of intravascular component such as serum because erythrocytes in the crude mincing extracts of the pneumonic group were much more than that of normal group. The ratio of palmitic acid to total fatty acids of lecithin of the supernate in each pneumonic group and also the proportion of total saturated fatty acids to total unsaturated fatty acids of lecithin of the supernate in pneumonia were significantly lower than those in normal.

On the other hand, the tension-concentration diagram of miscellaneous fatty acids showed that the more carbon number became and the higher saturated fatty acids became, the higher surface activity became. Although a major component of the pulmonary surfactants is dipalmitoyl-lecithin, this tension-concentration diagram shows that lecithin with other fatty acids except palmitic acid, particularly highly saturated fatty acids, had also high surface activity.

The tension-concentration diagram indicates that the surface activity must be discussed from the point of lipid and its fatty acid residue.

In pneumonic group the alteration of fatty acid composition of the supernate may be due to the contamination of serum because the
increase of erythrocytes indicates mixture of serum in the pneumonic lung extracts.

Hence, fatty acids of lecithin of serum and the supernate in each pneumonic group was analysed. Myristic, palmitoleic, stearic and oleic acid of lecithin of the lung surfactant obtained from each pneumonic group was not similar to those of serum lecithin.

Therefore, these alteration of fatty acids composition was not explained only by the contamination of serum component by the infection. The decrease of saturated fatty acids or the increase of unsaturated fatty acids is also a factor of the low surface activity in diplococcal and mycoplasmal pneumonia although the exdusion of serum component by the infection may be a factor of the low activity.

Judging from the tension-concentration diagram of various lipids, fatty acids composition is important for the surface activity. In the lung palmitic acid was incorporated dominantly. On the other hand, unsaturated fatty acids increased in the pneumonic lung. These data may show that the damaged or alternative selectivity of fatty acids occurred in the pneumonic lung and that its selection may be necessary to utilize these fatty acids for the energy. Tierney et al\(^{28}\) reported that half-life of polyenoic lecithin of the lung was longer than that of lecithin with palmitic acid. This report may support the increase of unsaturated fatty acids of lecithin of the damaged lung by infection. On the other hand, the administration of unsaturated fatty acid induced the increase of lipids in the lung but the administration of saturated fatty acid did not show the increase of lipids\(^{29}\).

This suggests that the lung utilizes unsaturated fatty acid, and it is thought that lipids with unsaturated fatty acids is advantageous for the energy of the lung itself. In fact, the administration of linoleic acid diminished the surface activity\(^{30}\). In pneumonia the selectivity of fatty acid takes advantage of the energy for the lung itself or the characteristic selectivity of fatty acids may be damaged like Greenstein's law in tumor tissues.

These unsolved problem must be researched by the other isotope experiment as palmitate-\(^{14}\)C and arachidonic acid-\(^{14}\)C. Furthermore, mixture of serum must be confirmed by \(^{131}\)I-albumin. These further experiment might resolve the mechanism of the increase of triglyceride or unsaturated fatty acids.

Furthermore, Kimura\(^{31}\) in our laboratory reported that triglyceride increase in the washing extracts from the pneumonic lung and triglyceride originated from the serum due to congestion. And he reported that triglyceride had the low surface activity. Hence, the increase of erythrocytes in this paper may be linear with the increase of triglyceride. On the other hand, Kimura and Mori\(^{31}\) showed the following result obtained from the isotope experiment. The incorporation of CDP-choline-1, 2-\(^{14}\)C was low in the lung slices of the pneumonic lobes
and also the incorporation of CDP-choline-1, 2-14C into the washing extracts was low in vivo in pneumonia. But the mechanism of increased triglyceride of washing extracts is not revealed by CDP-choline-1, 2-14C and the experiment of acetate-14C is necessary for its resolve. Although some problems remain unsolved, the result of CDP-1,2-14C experiment by KIMURA and MORI indicates that the low surface activity of the pneumonic lobes is not only due to the contamination of serum but also to the damaged metabolism of lipids of lung alveolar cells.

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