[Chem. Pharm. Bull.
31(11)4110—4115(1983)]

Lung Surfactants. III.[1] Correlations among Activities *in Vitro* *in Situ* and *in Vivo*, and Chemical Composition

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(Received December 21, 1982)

Correlations between surface activities *in vitro* and lung pressure–volume characteristics *in situ* or *in vivo* were examined with modified lung surfactants. Correlations were found between the value of the minimum surface tension and lung pressure–volume characteristics *in situ* or *in vivo*. The surface tension in compression at 60% surface area was also correlated with the lung pressure–volume characteristics *in situ* and *in vivo*.

Correlations between chemical components in the lung surfactant and its activities *in vitro*, *in situ* and *in vivo* were also examined. The content of disaturated phosphatidylcholine was correlated to the value of the minimum surface tension, the spreading rate and pressure–volume characteristics *in situ* and *in vivo*.

These results showed that the following *in vitro* criteria are essential for a lung surfactant to give normal pressure–volume characteristics to the mammalian lung *in situ* and *in vivo*; 1) a minimum surface tension of 10 dyn/cm or less, 2) a surface tension of 13 dyn/cm or less in compression at 60% surface area, 3) spontaneous spreading. Furthermore, we found that the content of disaturated phosphatidylcholine in the lung surfactant is an important determinant in relation to the above criteria.

Keywords——lung surfactant; modified lung surfactant; surface activity; spreading; lung pressure–volume characteristic; activity *in vitro–in situ–in vivo* correlation; disaturated phosphatidylcholine content-activity correlation

The activity of lung surfactant *in vitro* has generally been measured on a modified Wilhelmy surface balance.[2–4] Several criteria have been proposed for natural surfactant, including minimum surface tension, surface tension–surface area hysteresis and compressibility.[5–7] However, almost all of these criteria are presumptive and their importance has not yet been experimentally established.

The activity of lung surfactant has also been measured with the lungs of mammals. Ikegami *et al.* reported a procedure for measurement of the activity of lung surfactant with excised, lavaged rat lung *in situ*,[8] and Enhörning and Robertson reported a procedure with premature rabbit fetus *in vivo*.[9]

Although the activity of lung surfactant has been examined in various ways *in vitro*, *in situ*, and *in vivo*, mutual relations between these activities have not been well explored.

We previously reported modified lung surfactants possessing good activities *in vitro*, *in situ*, and *in vivo*.[1,10–12] Thus, we examined the relations between these activities with the modified lung surfactants. Several correlations between these activities were found, and several *in vitro* criteria were clearly established as being important for good lung pressure–volume characteristics *in situ* and *in vivo*.

Materials and Methods

Materials——1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Fluka (Buchs, Switzerland), and palmitic acid and tripalmitoylglycerol were purchased from Tokyo Kasei Co. (Tokyo). All other

chemicals were of reagent grade.

Chemical Analyses——The amounts of chemical components were determined by the methods described in the preceding paper.[12]

Modification of Lung Surfactant——The lung surfactant used was prepared from minced bovine lung by the method described previously.[12] The lung surfactant was lyophilized and dissolved in chloroform. Chloroform solutions of DPPC, palmitic acid and tripalmitoylglycerol were added in suitable proportions to an aliquot of the dissolved lung surfactant. The contents of disaturated phosphatidylcholine (DSPC), fatty acids and triacylglycerols were varied between 30—55, 3—13 and 3—13%, respectively. The protein content was reduced to 0.5—2.0% by this adjustment.

After these adjustments, the sample was suspended in 0.9% NaCl by the procedure described previously.[10,12] The concentration of the lung surfactant was adjusted to 50 mg phospholipids/ml of 0.9% NaCl.

Measurement of Surface Properties *in Vitro*——Surface activity was measured on a modified Wilhelmy surface balance at 37 °C in the way described in the preceding paper.[12] Spreading was also measured by the method described previously.[12]

Measurement of Lung Pressure–Volume Characteristics *in Situ*——Lung pressure–volume characteristics in the excised, lavaged rat lung were measured by the method of Ikegami *et al.*[8] with several modifications as described previously.[1]

Measurement of Lung Pressure–Volume Characteristics *in Vivo*——Lung pressure–volume characteristics in the premature rabbit fetus were measured by the method of Enhörning and Robertson[9] with several modifications as described previously.[12]

Results

Correlation between Activities *in Vitro* and *in Situ*

As Fig. 1 shows, a correlation was found between the value of minimum surface tension in the surface activity *in vitro* and the percent of total lung capacity (TLC_{30}) at a pressure of 5 cm H_2O in the excised, lavaged rat lung *in situ*. This percentage increased in proportion to the lowering of the minimum surface tension. The excised rat lung showed 40—60% of TLC_{30} at 5 cm H_2O before lavage. Most of the surfactants showing 40% of TLC_{30} and over at 5 cm H_2O showed a minimum surface tension of 10 dyn/cm or below. Other criteria for a natural surfactant *in vitro*, hysteresis area and compressibility, were not correlated to this percentage.

As shown in Fig. 2, the value of the surface tension at 60% surface area *in vitro* was

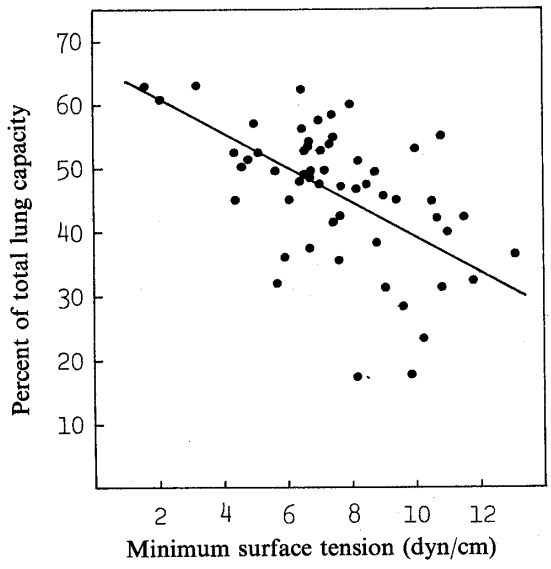

Fig. 1. Correlation between Value of Minimum Surface Tension *in Vitro* and Percentage of Total Lung Capacity at 5 cm H_2O in Rat Lung *in Situ*

$p<0.01$, $n=56$, $r=-0.575$, $y=66.6-2.76x$.

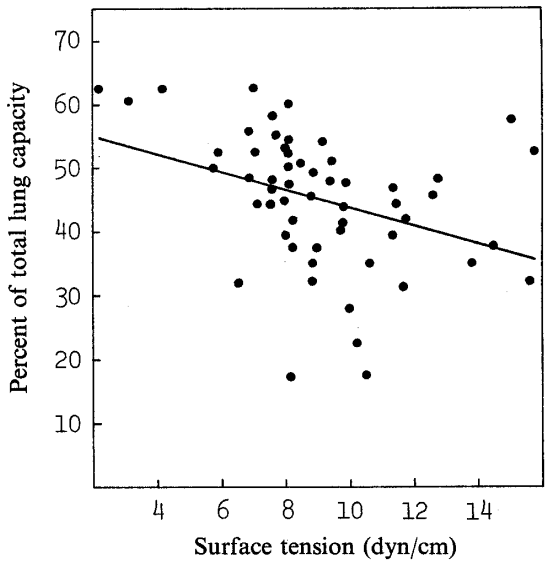

Fig. 2. Correlation between Value of Surface Tension at 60% Surface Area *in Vitro* and Percentage of Total Lung Capacity at 5 cm H_2O in Rat Lung *in Situ*

$p<0.01$, $n=56$, $r=-0.365$, $y=37.8-1.33x$.

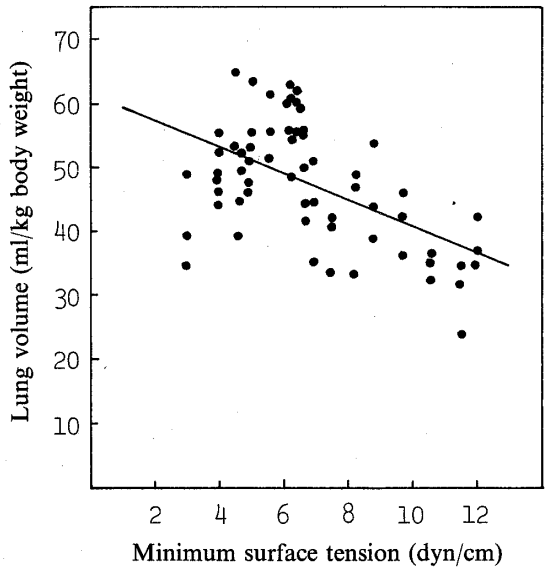

Fig. 3. Correlation between Value of Minimum Surface Tension *in Vitro* and Lung Volume at 5 cm H_2O in Premature Rabbit Fetus *in Vivo*

$p<0.01$, $n=63$, $r=-0.531$, $y=61.0-2.03x$.

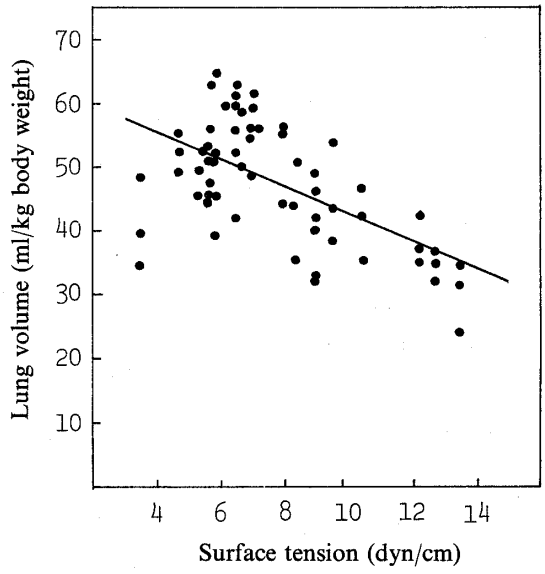

Fig. 4. Correlation between Value of Surface Tension at 60% Surface Area *in Vitro* and Lung Volume at 5 cm H_2O in Premature Rabbit Fetus *in Vivo*

$p<0.01$, $n=63$, $r=-0.587$, $y=63.5-2.11x$.

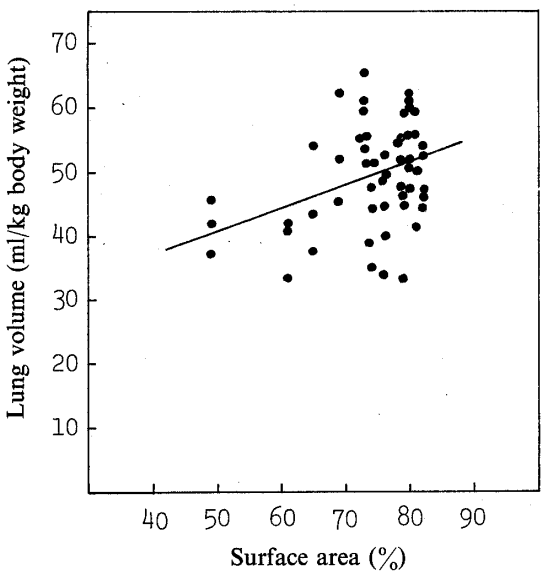

Fig. 5. Correlation between Percentage of Surface Area at 10 dyn/cm *in Vitro* and Lung Volume at 5 cm H_2O in Premature Rabbit Fetus *in Vivo*

$p<0.01$, $n=54$, $r=0.382$, $y=22.7+0.36x$.

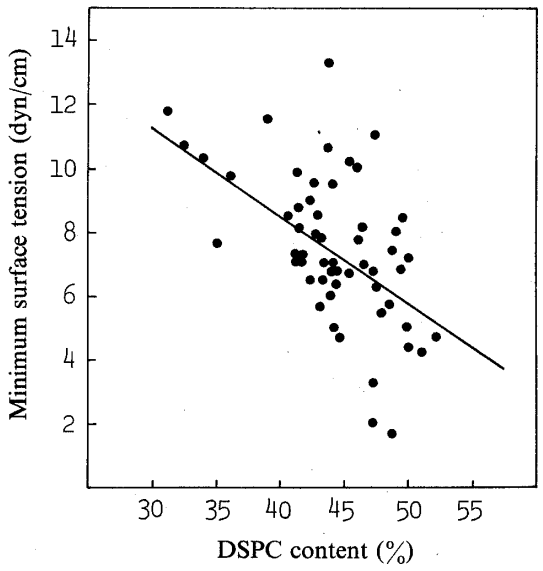

Fig. 6. Correlation between DSPC Content in Lung Surfactant and Value of Minimum Surface Tension *In Vitro*

$p<0.01$, $n=56$, $r=-0.506$, $y=19.3-0.27x$.

correlated to this percentage. Most of the surfactants showing 40% of TLC_{30} and over at 5 cm H_2O showed a surface tension of 13 dyn/cm or less in compression at 60% surface area. The percentage was not correlated to the value of the maximum surface tension.

Correlation between Activities *in Vitro* and *in Vivo*

As Fig. 3 shows, a correlation was found between the value of the minimum surface tension *in vitro* and the lung volume at 5 cm H_2O in the premature rabbit fetus *in vivo*. The

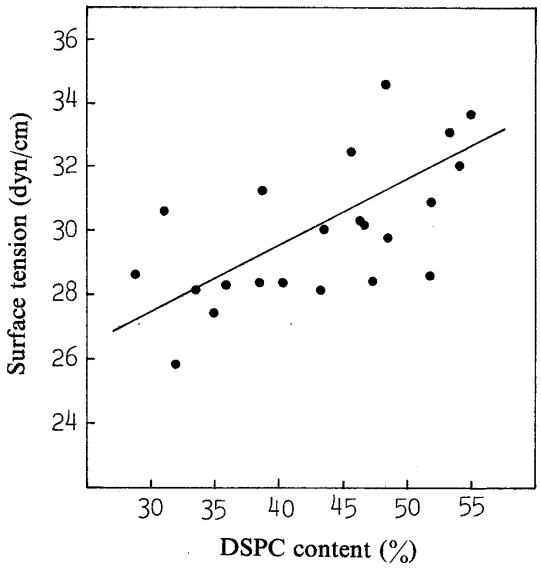

Fig. 7. Correlation between DSPC Content in Lung Surfactant and Spreading Rate

The value of surface tension at 30 s after sample layer formation was used as the spreading rate.
$p<0.01$, $n=22$, $r=0.660$, $y=21.9+0.186x$.

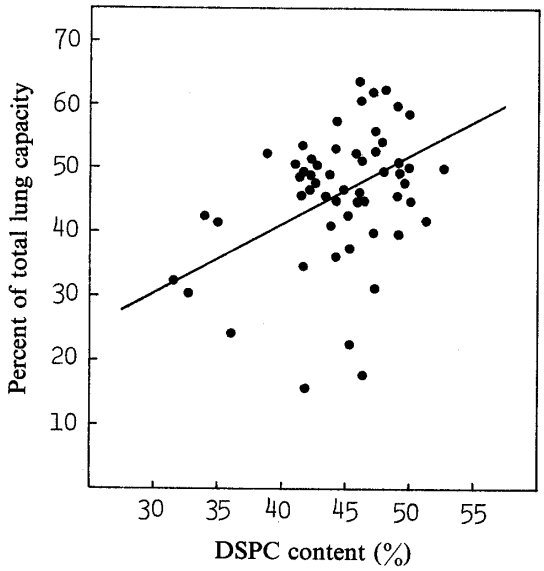

Fig. 8. Correlation between DSPC Content in Lung Surfactant and Percent of Total Lung Capacity at 5 cm H_2O in Rat Lung *in Situ*

$p<0.01$, $n=56$, $r=0.417$, $y=-1.19+1.06x$.

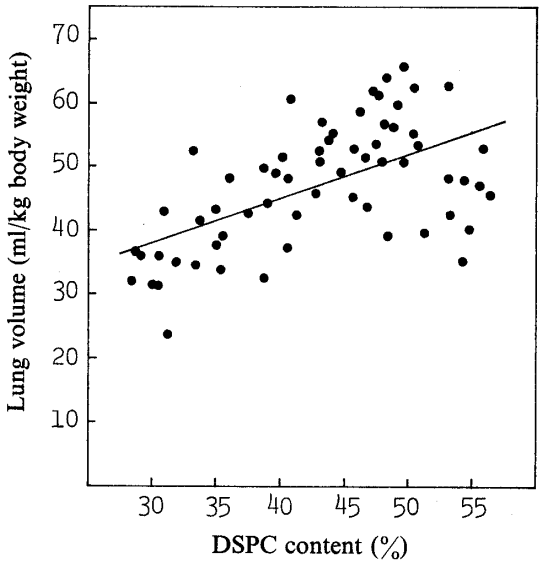

Fig. 9. Correlation between DSPC Content in Lung Surfactant and Lung Volume at 5 cm H_2O in Premature Rabbit Fetus *in Vivo*

$p<0.01$, $n=63$, $r=0.588$, $y=17.7+0.68x$.

lung volume increased in proportion to the lowering of the minimum surface tension. The lung volume at 5 cm H_2O in the mature rabbit fetus (gestation = 30 d) was 35—55 ml/kg body weight. Most of the lung surfactant giving 35 ml/kg and over as the value of the lung volume to the weight of the premature fetus showed a minimum surface tension of 13 dyn/cm or below *in vitro*. Other criteria for natural surfactant *in vitro*, hysteresis area and compressibility, were not correlated to the lung volume.

The value of surface tension at 60% surface area was correlated to the lung volume as shown in Fig. 4; at least 35 ml/kg lung volume at 5 cm H_2O in the premature rabbit fetus *in vivo* was obtained with surfactant giving more than 13 dyn/cm surface tension *in vitro*.

The correlation between the percent of the surface area at a surface tension of 10 dyn/cm *in vitro* and the lung volume at 5 cm H_2O *in vivo* is shown in Fig. 5. The lung volume is proportional to the percent of the surface area; lung surfactant giving a large lung volume to

the premature fetus also gave a large value for the percent of the surface area at 10 dyn/cm.

Correlation between DSPC Content and Activities *in Vitro*, *in Situ* and *in Vivo*

As Fig. 6 shows, the content of DSPC in the lung surfactant was correlated to the value of the minimum surface tension *in vitro*. Almost all of the lung surfactants containing 35% DSPC and over showed a minimum surface tension of 10 dyn/cm or less.

The content of DSPC was also correlated with the spreading rate as shown in Fig. 7. The values of the surface tension at 30 s after sample layer formation were between 25—35 dyn/cm and the lung surfactants containing large amounts of DSPC showed high surface tensions in this range, that is, the surfactants showed slow spreading rates.

The content of DSPC was further correlated to the percent of TLC_{30} at 5 cm H_2O *in situ* and the lung volume at 5 cm H_2O *in vivo* as shown in Figs. 8 and 9, respectively. Almost all of the lung surfactants containing 40% DSPC and over showed 40% of the TLC_{30} *in situ* and 45 ml/kg lung volume and over *in vivo*.

Discussion

The surface tension–surface area diagram on a modified Wilhelmy surface balance and the spreading rate on a subphase solution have been measured in attempts to characterize the surface activity of lung surfactant.[3,13,14] However, these activities are qualitative ones, since no quantitative method of measuring surface activity has yet been found. The following properties have been given as essential criteria for natural surfactant on the basis of these qualitative results and others: (1) a minimum surface tension of 10 dyn/cm or below,[5-7] (2) spontaneous spreading on a subphase of 0.9% NaCl,[13,14] (3) the presence of surface tension–surface area hysteresis,[5] (4) a compressibility of 0.09 cm/dyn or less at 10 or 15 dyn/cm at 37 °C.[5,7]

Even if a surfactant satisfies these criteria *in vitro*, its lung pressure–volume characteristics with the mammalian lung would have to be evaluated, because correlations have not yet been established between the activities of lung surfactant *in vitro* and *in vivo*. The importance of these criteria *in vitro* and the relation of these criteria to the activities *in situ* and *in vivo* thus remains unclear. If we can obtain correlations between the activities *in vitro* and *in vivo*, it might be possible to predict the physiological activity from the activity *in vitro*.

The value of the minimum surface tension *in vitro* was correlated to the percent of TLC_{30} at a pressure of 5 cm H_2O *in situ* and the lung volume at 5 cm H_2O *in vivo*. It was necessary for a lung surfactant to show a minimum surface tension of 10 dyn/cm or less *in vitro* in order to give normal lung pressure–volume characteristics *in situ* and *in vivo*, in accordance with the conventional view that a minimum surface tension of 10 dyn/cm or less is an important criterion for a natural surfactant.

All the surfactants used showed spontaneous spreading and surface tension was lowered to 25—35 dyn/cm within 30 s after sample layer formation on 0.9% NaCl. This spreading rate is much faster than those of already known surfactant preparations, *e.g.* 40 min.[3]

The content of DSPC in the lung surfactant was correlated to the values of the minimum surface tension and spreading rate *in vitro*, the percentage of TLC_{30} at 5 cm H_2O *in situ*, and the lung volume at 5 cm H_2O *in vivo*. These results supported our previous conclusion that the content of DSPC is related to the surface properties of lung surfactant *in vitro* and to the physiological activities *in situ* and *in vivo*.[10,15]

Correlations were not found between the hysteresis area or the compressibility at 10 or 15 dyn/cm *in vitro* and pressure–volume characteristics *in situ* or *in vivo*. The areas were too large and the values of the compressibility were too low for correlations, if present, to be easily detectable. We found that 13 dyn/cm surface tension or less in compression at 60% surface

area *in vitro* was an important criterion for normal physiological activities *in situ* and *in vivo*. All the surfactants satisfying this criterion showed a large hysteresis area, and a compressibility of 0.03 cm/dyn or less at 10 dyn/cm. The values of the compressibility were far lower than 0.09 cm/dyn.[5,7] Thus we concluded that these criteria are of secondary importance for natural surfactants.

In addition to the usual criteria for natural surfactants, we examined correlations between various parameters of surface activity *in vitro* and physiological activities *in situ* or *in vivo*. We found that a surface tension of 10 dyn/cm and below at a large percent of surface area is an essential criterion for good physiological activities. Almost all of the established surfactant preparations showed a minimum surface tension of 10 dyn/cm and below at 20% of surface area.[3,16] Our surfactant showed a minimum surface tension of 10 dyn/cm or less at a far smaller compression than those surfactants. The faster the velocity of surface tension decrease in the compression cycle *in vitro*, the better the physiological activities of lung surfactant *in situ* and *in vivo* were.

We think that the following activities *in vitro* are minimum essential criteria for a surfactant to give normal lung pressure–volume characteristics *in situ* and *in vivo*: 1) a minimum surface tension of 10 dyn/cm or less, 2) a surface tension of 13 dyn/cm or less in compression at 60% surface area, 3) spontaneous spreading. Furthermore, we found that the DSPC content in the lung surfactant was correlated with these surface and physiological activities *in vitro* and *in vivo*. We previously reported that fatty acids, triacylglycerols and a protein were also involved in these activities.[1,10,15]

Acknowledgement The authors are grateful to Dr. Tetsuro Fujiwara of Iwate Medical University for his helpful advice and encouragement.

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