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Shear Viscosity of the Surfactant from Dog Lungs

Viscosidad de superficie del surfactante de pulmón de perro

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1. Surface viscosity (η_S) was measured in the pulmonary lavage fluid obtained from 40 mongrel dogs.
2. Two experimental methods were employed to determine surface viscosity: a) Fourt's "torsion pendulum" procedure; and b) the "viscous traction" viscosimeter. A satisfactory correlation (r = 0.88) between these two methods was obtained.
3. The mean value for surface viscosity of the pulmonary lavage fluid was 3.8 x 10^{-2} surface-poise (s.p.) with the torsion pendulum procedure, and 4.3 x 10^{-2} s.p. with the viscous traction viscosimeter.
4. The implications of surface viscosity in pulmonary mechanics is discussed, as well as the effects of blood plasma addition to the pulmonary lavage fluid. A significant increase of surface viscosity could be observed when blood plasma was added to the hypophase, in concentrations greater than 0.1‰. This increase in surface viscosity might be of importance when related to the augmented respiratory work during the evolution of pulmonary edema.

Mammalian pulmonary alveoli are lined by a surface-active substance, the pulmonary surfactant (1), whose chemical composition and the surface tension have been thoroughly investigated (2, 3). Brown *et al* (4) have measured its surface compressibility coefficient and compared it with those of blood plasma. Meban (5) has determined the "dilational viscosity" of surfactant films and has extrapolated his *in vitro* results to the *in vivo* conditions. He has also analyzed the influence of surface viscosity on the mechanical efficiency of the lungs. The same author (6) has measured the "shear viscosity" of pulmonary lavage fluid from 10 human cadavers 3 to 6 hours after death and after removal of the exfoliated cells by centrifugation at 800 x g for 7 min at 4°C. The resulting supernatant was centrifuged at 10.000 x g for 7 min, in order to remove the heavier cytoplasmic debris; finally, 30 ml of this second supernatant was diluted to a volume of 300 ml, which the author termed "cell free lung lavage".

In the present study we investigated the shear viscosities of the pulmonary surfactant and of blood plasma from dogs, and further analyzed its possible effects on pulmonary mechanics *in situ*. For this purpose we assumed that the *in vitro* results can be extrapolated to the *in vivo* situation, since the knowledge of the shear viscosity allows one to know the conditions under which the surfactant can flows, i.e., its drainage from the alveoli to the bronchiolus, as well as its influence on respiratory work, both under normal and pathological conditions. The extrapolation to the *in situ* is validated by the fact that, in this study, pulmonary lavage fluid was used instead of a chemical surfactant. The temperature difference, 20°C *in vitro vs* 37°C *in situ*, is also of great importance. Meban (6) has found that the surface viscosity ($g.s.^{-1}$ x 10^4) of this dilute film decreased from 12.0 ± 0.2 units at 25°C to 2.8 ± 0.04 units at 37°C (see his Table 2, p. 223). This temperature effect on the surface viscosity is more pronounced than the equivalent viscosity changes in distilled water, since in the latter case the viscosity varies from 1.00 centipoise (c.p.) at 20°C to 0.6947 c.p. at 37°C (7).

It is interesting to note that shear viscosity is equivalent to the canonical three dimensional viscosity, since all fluids poses definite resistance to change of form due to the internal friction among molecules moving at different speeds. Thus, the

force (F) necessary to move a linear element in relation to another is a function of the velocity gradient (dv/dx), the length of the element (1), and of the surface viscosity (η_S) or of the friction between molecules in motion:

$$F = \eta_S \cdot 1 \cdot \frac{dv}{dx} \qquad (1)$$

$$[\eta_S] = \frac{F}{1 \cdot \frac{dv}{dx}} = \frac{M \cdot L \cdot T^{-2}}{L \cdot (L \cdot T^{-1}) \cdot (L^{-1})} = [M \cdot T^{-1}] \qquad (2)$$

When these results are expressed in cgs units, the dimension corresponds to g · seg $^{-1}$, which is equivalent to "surface poise" (s.p.).

MATERIALS AND METHODS

Forty mongrel dogs, whose weighs ranged from 8.6 to 20.5 kg, were anesthetized with sodium pentobarbital 30 mg/kg (intraperitoneal) and exsanguinated by inserting a cannula into the femoral artery. After thoracotomy, the pulmonary vascular bed was thoroughly perfused with isotonic saline (NaCl 8.5%) while the pulmonary perfusion pressure was maintained below 10 cm H_2O. For this purpose the superior vena cava was cannulated after the ligature of the remaining veins leading to the right auricle and the aorta was transsected to reduce the outflow resistance almost to zero. Both lungs were then excised and 200 to 250 ml of the isotonic saline solution was introduced into the trachea; of those amounts, 70 to 75% of the lavage fluid could be recovered. Five minutes later the intrapulmonary fluid was recovered and filtered through four layers of degreased gauze to eliminate the mucus that comes from the airways. Samples of this pulmonary lavage fluid were employed for the measurement of surface tension (γ) and of surfactant viscosity (η_S).

The surface active material was added to the saline solution inside appropriate troughs, that were made either of hydrophobic material or covered with a thin layer of solid paraffin After a 30 minute stabilization period, the measurements of surface tension (γ) and of surface viscosity (η_S) were initiated.

Finally, in 10 experiments, the influence of the addition of different amounts of canine blood plasma to the hypophase was studied. The final plasma concentrations were 0,1, 1,0, and 10‰. The surface viscosities of the pulmonary lavage fluids were measured as in the control experiments.

A. *Surface tension measurements.* Two techniques were employed:

1. *Wilhelmy's method* in which the amount of pull exerted by the surface active material on a platinum plate introduced into the liquid contained in the pyrex dish is measured; and

2. *Langmuir's method,* in which the monomolecular film is formed on the surface of the liquid inside a rectangular trough and limited by a movable barrier. After a stabilization period it is possible to measure the surface tension, both when the monolayer is expanded to 100% of the area (γ max) and when the surface film is compressed to 20% of the original area, at this point the surface tension is designated as gamma minimum (γ min). Since the latter value is considered as an "activity index" of the surfactant, only when γ– min was less than 10 dynes/cm was the pulmonary lavage liquid considered as satisfactorily active for the purpose of the present study.

B. *Shear viscosity measurements*

Since this property of the pulmonary surfactant has scarcely been investigated (6), it seemed mandatory to employ two different techniques in order to compare the two and to evaluate their accuracy.

1. In the *Fourt's "torsion pendulum"* method (Fig. 1) the cylinder of the pendulum as a set of transversal platinum wires attached in its lower part. These are 0.5 cm, 1.0 cm and 4.0 cm long.

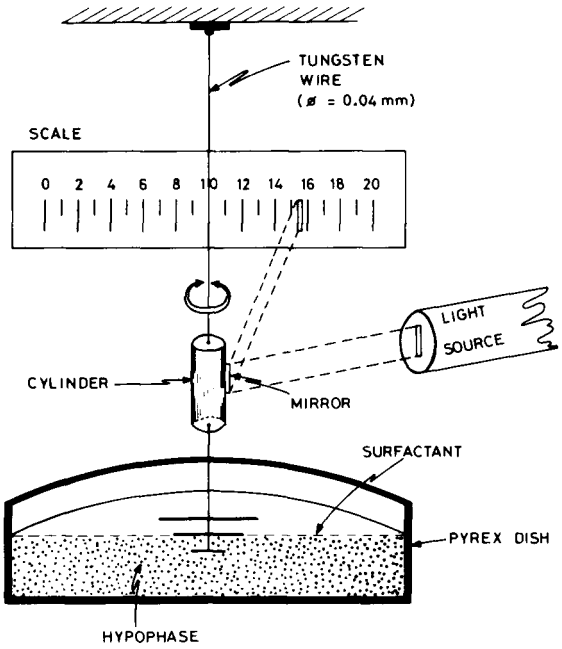

Fig. 1: Diagram of Fourt's torsion pendulum procedure for shear viscosity measurements of the pulmonary surfactant. For description see text.

In order to proceed with the measurement of the surface viscosity, one of these transversal platinum wires were put in contact (tangential) with the surface of the monolayer proper. The pendulum was subjected to a rotatory displacement and then released so that a spontaneous oscilatory movement was initiated, and whose subsequent amplitudes could be measured on a scale by means of a beam of light reflected on a mirror attached to the cylinder of the pendulum. Control measurements with either distilled water or with saline solutions showed a reduced damping effect on the amplitude of these oscillations, whereas the damping phenomenon was notorius when the monolayer had a marked surface viscosity.

The surface viscosity (η_S) was calculated in accordance with Fourt's equation, as modified by Trapeznikov (8, 9):

$$\eta_S = \frac{9.2\ I}{1^2} \left(\frac{\lambda p}{T_p} - \frac{\lambda 0}{T_0} \right) \qquad (3)$$

Where:

I = momentum of the pendulum, which in the present case was 3.3545 g • cm^2;

l = length of the transversal wire employed;

λ = the logarithmic decrement of the oscillations, where

$$\lambda = \frac{1}{n-1} \log \frac{\theta_1 - \theta_i'}{\theta_n - \theta_n'} \qquad (4)$$

n = number of complete oscillations;

θ and θ' = two successive measurements of the amplitude;

T = period of one complete oscillation.

The subindex "o" means that the surface of the liquid was clean, whereas the subindex "p" indicates that the surface of the liquid was covered by the monolayer of the pulmonary surfactant

2. The second method is based on *"viscous traction"*. This surface viscometer consists of two concentric rings of stainless steel (Fig. 2), whose diameters are 12.5 cm and 11.6 cm at the free edges, respectively. These concentric rings were suspended in such a way that the free edges got exactly in contact with the surface of the liquid to be studied. The pyrex dish (diameter = 14 cm) filled with liquid was placed on a rotatory disc –one revolution in 40 sec activated by means of vibration– free motor. Small talc particle was carefully placed on the surface of the circular channel, between the two concentric rings, in order to measure its displacement measure, and consequently to determine its velocity. This was done first in a clean liquid channel distilled water and afterwards when the liquid (hypophase) was covered by the surface active monolayer. The difference between both velocity measurements corresponds to the delay caused by the viscosity of the surface film. It should be added that this instrument must be calibrated with substances of well known surface viscosities, such as stearic or palmitic acids in different concentrations.

All surface tension and viscosity measurements were performed in *ad hoc* chambers, whose ambiental temperature was 20 ± 0.5°C.

The pyrex dishes, as well as the viscometer rings, were carefully degreased with chromic acid, and afterward cleansed with abundant distilled water. Those parts of the pyrex dish in contact with the surfactant were covered –prior to each series of measurements– with a coating of solid paraffin, by immersion of the free border in melted wax.

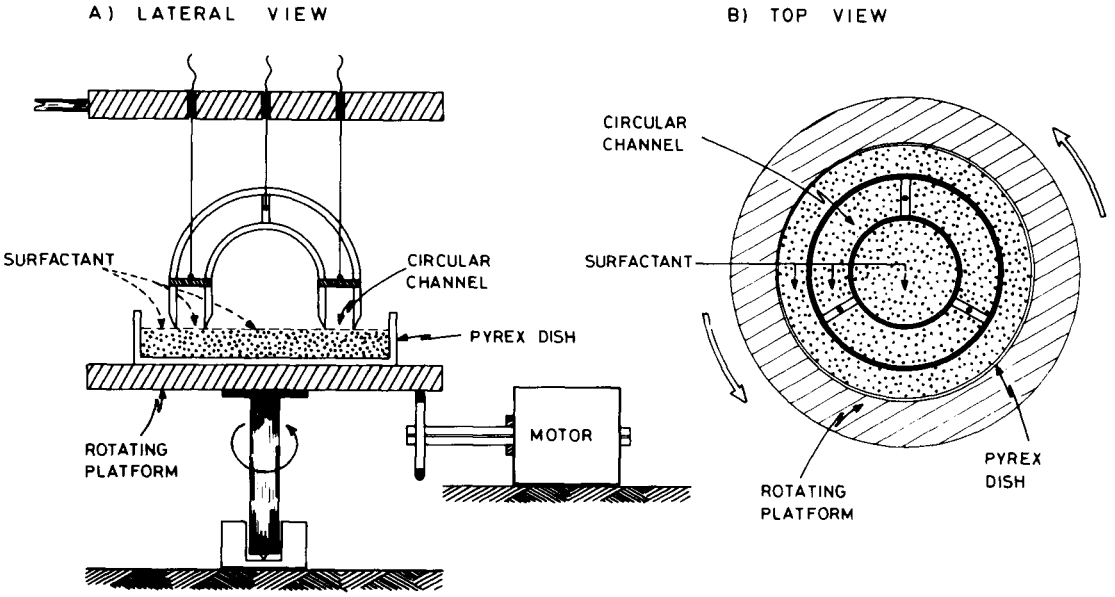

Fig. 2: Schematic drawing of the experimental apparatus for shear viscosity-determinations in accordance with the viscous traction method. For description see text.

RESULTS

The values obtained from the comparative viscosity measurements (η_S) are shown in Fig. 3, where the "torsion pendulum" results (X) were plotted against the "viscous traction" values (Y). A satisfactory correlation between both methods was found (r = 0.88). Nevertheless, a small shift of the regression line could be observed (Fig. 3), due to the fact that the results of the "viscous traction" method (Y) are displaced by 3 x 10^{-2} s.p. units in the ordinates with respect to the "torsion pendulum" method (X).

The mean values and the standard deviations of surface tension (γ) and surface viscosity (η_s) of the pulmonary lavage fluid obtained from 40 mongrel dogs are shown in Table 1.

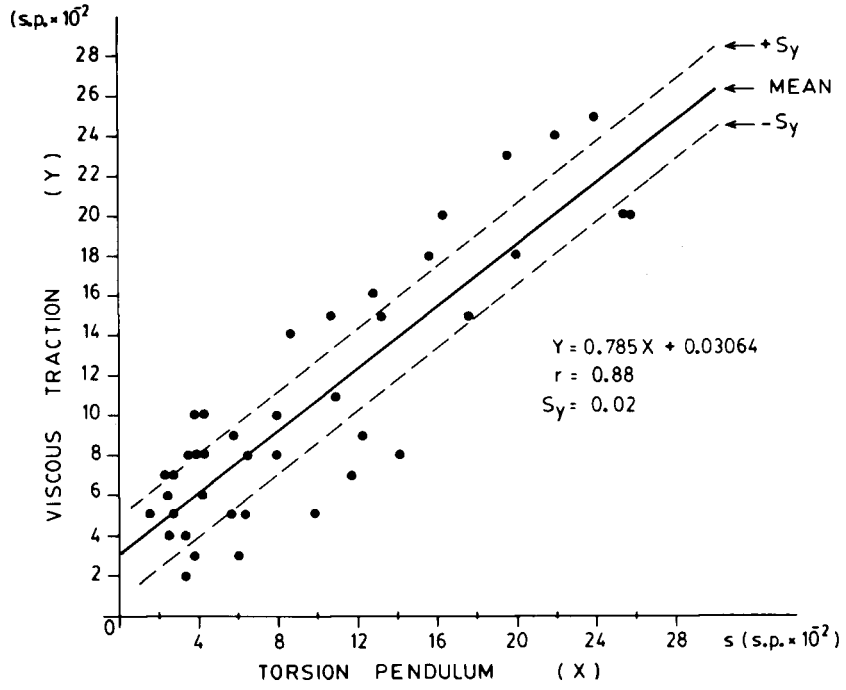

Fig. 3: Correlation between the experimental results obtained with the torsion pendulum method (X) and the viscous traction procedure (Y) in pulmonary lavage fluids from 40 mongrel dogs.

TABLE 1

Statistical analysis (mean ± standard deviation) of the surface tension (γ max and γ min), as well as the corresponding values for the surface viscosity (η_S), which were obtained with both methods, the "torsion pendulum" and the "viscous traction"

Surface Tension (dynes · cm^{-1})		Surface Viscosity (s.p. x 10^{-2})	
γ max.	γ min.	Pendulum	Traction
51.5 + 3.6	7.0 ± 2.2	9.6 ± 7.1	10.6 ± 6.3

The maximum surface viscosity which was obtained by means of these two procedures was 25.4 x 10^{-2} s.p.

It is also interesting to note that no correlation was observed between the surface viscosity (η_S) and the surface tension (γ) of the pulmonary lavage fluids from dog lungs.

Finally, the effect of canine blood plasma additions to the hypophase was also investigated (Table 2). The analysis of variance of the values shown in Table 2 yielded highly significant differences ($p < 0.0005$) between the control values of the surface viscosity and those obtained after the addition of blood plasma to the hypophase. Furthermore, when the effects of different final plasma concentrations were compared, the difference was significant ($p < 0.01$) between 0.1‰ and 1‰, as well as between 0.1‰ and 10‰. Nevertheless, between plasma concentration of 1‰ and 10‰, the effects on the surface viscosity was not significant ($p > 0.01$).

TABLE 2

Effect of blood plasma additions on the surface viscosity of the pulmonary lavage fluid obtained from 10 mongrel dogs. Mean values ± standard deviation

Surface viscosity (η_S) s.p. x 10^{-2}	Surface viscosity after the addition of blood plasma to the hypophase		
	0.0‰	1‰	10‰
12.1 ± 7.3	17.1 ± 7.5	172.7 ± 159.1	61.6 ± 20.2

DISCUSSION

The surface viscosity of pulmonary lavage fluids, with the exclusion of the mucus from the air ways, was measured directly. The aim was to determine the shear viscosity of the pulmonary surfactant under conditions resembling those *in situ*, since the pulmonary lavage fluid contains practically all the components which normally are found in the alveolar lining. Other authors have measured surface viscosities of "pulmonary extracts" (5, 6), but they have not measured the surface activity of these extracts. For this reason, we first determined γ-min, in order to be sure that our lavage fluid contained enough active surfactant. We then added a known amount of pulmonary lavage to the saline in the pyrex dish in order to obtain a γ-max similar to the value obtained with the surface tension balance, whose γ min $\leqslant$ 10 dynes/cm. The Wilhelmy "traction method" was employed to measure γ max of the monolayer which subsequently was subjected to the surface viscosity measurements (η_S).

In monomolecular films of surfactants a correlation between surface tension (γ) and surface viscosity (η_S) has been described (10). Nevertheless, this correlation was absent in our experiments due probably to the fact that the pulmonary lavage fluid contained not only surface-active substances, but also exfoliated cells, cytoplasmic debris, bronchial secretions, among other contaminants. Furthermore, from a physical point of view, *surface tension* (γ) is a "static" phenomenon, which is due to molecular attractions on the surface of separation between two liquids, or between the air-liquid interphase at the pulmonary alveoli. This tension is expressed in "dynes/cm". Contrariwise, *surface viscosity* (η_S) is a "kinematic" property, which results from a tangencial force per unit length, exerted by molecules of the monolayer upon adjacent molecules of the surfactant. In the first case we are dealing with a surface tension, and in the second, with the internal friction between molecules in motion.

"Surface viscosity" is a property inherent to the friction between the molecules on the surface lining layer, and for this reason we may assume that this surface viscosity is of rather little importance with regards to normal pulmonary work. Meban (6) found that at a surface coverage of less than 2.5 cm^2/g extract, the value of η_S increased sharply Nevertheless, one should take into account that during normal breathing the changes of the alveolar area are relatively small, and therefore small changes of the surfactant should not significantly affect shear viscosity. As mentioned above, η_S is a property which depends mainly on intermolecular friction. On the other hand, the surface pressure is responsible for the expansion of the monomolecular layer and consequently it is necessary to perform a certain amount of work to keep the film at a constant area. When the original alveolar area is reduced it is also necessary to take into account the velocity of the area reduction process. In this particular case η_S will the main factor in determining the force which should be applied to move the surface molecules at a given rate (see eq. 1).

If we now intend to extrapolate from our *in vitro* results to the normal conditions *in situ*, it follows that surface viscosity (η_S) does not represent an appreciable

or an additional resistance during normal breathing, since all the η_S figures are relatively low ($\leqslant 25.4 \times 10^{-2}$ s.p.); in addition, the alveolar area changes are relatively small during the normal respiratory cycle. But, if we assume for a moment that surface viscosity –under abnormal conditions– could be markedly increased, then the following alternative efects should be taken into account:

a) the reduction of the alveolar area –even at normal speed– means that the applied force should be increased;
b) if the applied force is unable to maintain a given velocity of the reduction of the alveolar area, then the expiratory period must be lengthened; and
c) when an increase of the duration of the expiratory phase is also impossible, then the normal expiratory alveolar surface will not be attained, and consequently the functional residual capacity of the lungs will be increased, as well as the transpulmonary expiratory pressure. Irrespective of the relative importance of the alternatives discussed above, they all seem to imply an increase of the transpulmonary pressure, particularly if the duration of the pressure gradient is taken into account, i.e., the magnitud of the pulmonary "action" (force x time), which should lead to an increase of the respiratory work. Some preliminary experiments seem to indicate that this situation may arise if blood plasma appears in the alveolar space, competing with the surfactant which is normally present in the air-liquid interphase. When 1-4 μl of plasma were added to the saline trough (whose area is about 50 cm^2), surface viscosity increased proportionally, reaching a value of 100×10^{-2} s.p. or 1.0 surface-poise, i.e., four times the maximum which was obtained with the surfactant (see Fig. 4). It is therefore likely, that surface viscosity might be an important factor in the respiratory work during pulmonary edema, a pathological condition where blood plasma transudes from the pulmonary capillaries into the alveolar space.

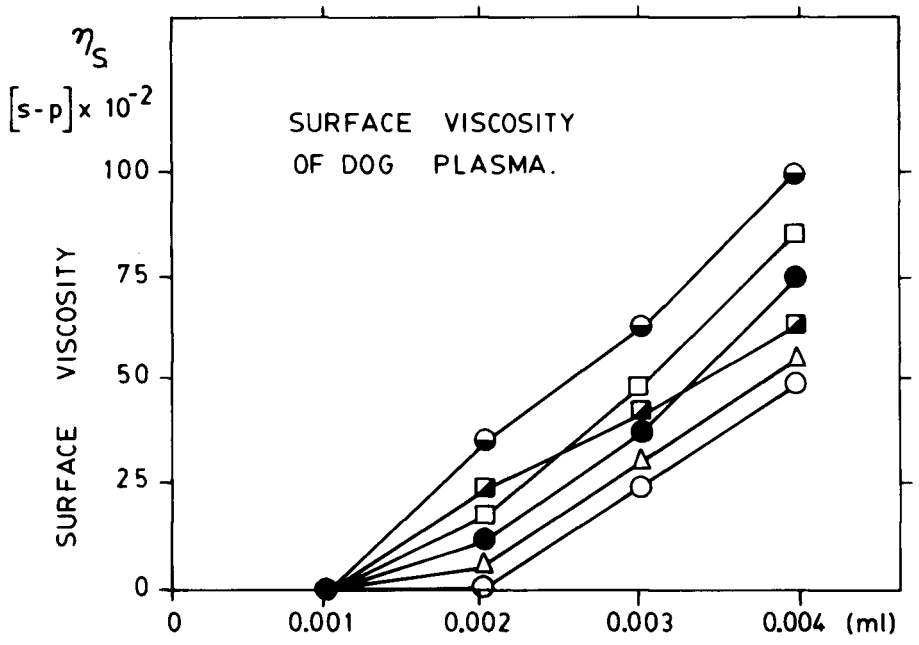

Fig. 4: Effect of increasing quantities (ml) of added dog plasma (abscissa) to the distilled water in the hypophase on shear surface viscosity.

In order to elucidate the possible influence of blood plasma components on the surface viscosity of the air-liquid interphase at the pulmonary alveoli, we made some preliminary observation by means of the addition of canine blood plasma to the hypophase of the pulmonary lavage fluid. When the plasma concentration in the hypophase was increased from 0.1‰, to 1‰, and finally to 10‰, it was observed that surface viscosity (η_S) increased in all instances (Table 2) when compared with control values. Nevertheless, the surface viscosity decreased at a plasma concentration of 10‰, in comparison with the 1‰, concentration. This anomalous behavior agrees with McRitchie's (11) observations which were made with mixtures of lecithin and polyamines, in which an initial increase was followed by a decrease of the surface viscosity when the concentrations of the mixtures were progressively augmented.

Our experiments in which blood plasma was added to the hypophase of the pulmonary lavage fluid should be considered only as preliminary, since the purpose of this study was only to evaluate the effect of blood plasma on the hypophase of the pulmonary surfactant, because during the evolution of a pulmonary edema the transudation of plasma from the inside of the pulmonary capillary network might affect the surface viscosity, and consequently the respiratory work under this pathological condition.

Finally, it must be noted that we were able to find a satisfactory correlation between the surface viscosity figures obtained with the Fourt's "torsion pendulum" method, and with the "viscous traction" viscometer. This finding is important because Fourt's viscometer is easier to build and to handle than the viscous traction procedures. Nevertheless, one could assume from a theoretical point of view, that the tangential contact with platinum wire of the torsion pendulum should affect the continuity of the monolayer, but the stability of the surfactant monolayer is a kinetic phenomenon, and for this reason it is likely that small disturbances produced by the rotatory movement of platinum wire in reality should not modify the stability of the surface film nor its shear viscosity

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