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Lubricating effect of sialomucin and hyaluronan on pleural mesothelium

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Running head: PLEURA LUBRICATION BY SIALOMUCIN AND HYALURONAN

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Abstract

Coefficient of kinetic friction (μ) between rabbit visceral and parietal pleura, sliding in vitro at physiological velocities and load, increases markedly after blotting mesothelial surface with filter paper; this increase is only partially reduced by wetting blotted mesothelium with Ringer solution. Given that mesothelial surface is covered by a thick coat with sialomucin and hyaluronan, we tested whether addition of sialomucin or hyaluronan solution after blotting lowers μ more than Ringer alone. Actually, these macromolecules lowered μ more than Ringer, so that μ was no longer significantly higher than its preblotting value. Moreover, Ringer addition, after washout of macromolecule solution, increased μ, in line with their dilution. These findings indicate that mesothelial blotting removes part of these molecules from the coat covering mesothelial surface, and their relevance for pleural lubrication. Transmission electron micrographs of pleural specimens after mesothelial blotting showed that microvilli were partially or largely removed from mesothelium, consistent with a substantial loss of macromolecules normally entrapped among them.

Keywords: Friction coefficient, hyaluronan, microvilli, pleural lubrication, phospholipids, sialomucin

1. Introduction

In 1973 Andrews and Porter by transmission electron microscopy showed a coat, probably acid mucopolysaccharide in nature, entrapped among the microvilli of rat pleura and peritoneal mesothelium fixed by vascular perfusion. They pointed out that this coat should be important for lubrication of the mesothelial surfaces. Moreover, staining with ruthenium red, they showed many fine polyanionic strands radiating from microvilli, interconnected with each other and adjacent microvilli. Wang (1974) by transmission electron microscopy noticed that colloidal iron stains the surface of rabbit pleural mesothelium, and that this staining persists after hyaluronidase treatment, but is abolished by neuroaminidase treatment. This indicates the presence of sialic and other mucoproteins in the glycocalyx of the pleural mesothelium (Wang, 1985). More recently, Ohtsuka et al. (1997) showed, with either cationic colloidal iron or limax flavus lectin, a coat of sialomucin with many negative charges on the mesothelial surface of mouse pleura, pericardium, and peritoneum. This coat is much thicker than the usual glycocalyx occurring on cell plasma membrane. Hyaluronan might also occur in this coat of the mesothelial surface, because it has been found in small concentration both in the pericardial (82 μg/ml, Honda et al., 1986), and pleural (0.73 μg/ml, Wang and Lai-Fook, 1998) liquid of rabbits. However, Ohtsuka et al. (1997), like Wang (1974), found that hyaluronidase did not affect colloidal iron staining of their mesothelium specimens, while neuraminidase removed this staining.

In 1982 Hills et al. showed that surface active phospholipids, extracted with chloroform-methanol from pleural washing, lowered the coefficient of kinetic friction (μ) of cotton yarn. They pointed out that these phospholipids may be important for boundary lubrication of the pleural surfaces. Similar phospholipids were also found on the surface of the pericardium (Hills and Butler, 1985), and of joints (Hills, 1989). Extracts from joints were placed on quartz plates, and μ was measured under a normal load, and various sliding velocities: it was ~ 0.005, and did not change with sliding velocity. Similar measurements of μ were performed on phospholipids extracted from blotted visceral pleura, and left overnight on the plates: μ was 0.056, i.e. higher than that of joint

phospholipids, likely because of a thinner layer of phospholipids, due to their smaller amount per unit surface (Hills, 1992). Finally, transmission electron microscopy of visceral pleura, fixed with tannic acid and osmium tetroxide, showed 5-7 osmiophilic layers on the mesothelial surface, likely due to phospholipid lamellar layers (Hills, 1992). On the other hand, the spatial and functional relationships between phospholipid layers and mucopolysaccharide coat on the mesothelial surface under physiological conditions are not known.

D'Angelo et al. (2004) measured the frictional force of rabbit parietal pleura sliding against visceral pleura in vitro while oscillating at physiological velocities and amplitudes under physiological load; these measurements were also made on peritoneum samples. They found that the frictional force is directly proportional to the load, and independent of velocity of sliding and nominal contact area, consistent with boundary lubrication. Indeed, although pleural liquid or Ringer bicarbonate solution was present between the mesothelial surfaces, μ did not change with the change in sliding velocity. The values of μ for the pleural mesothelium was 0.019 ± 0.002 with pleural liquid between the mesothelial surfaces, and 0.027 ($P<0.01$) with Ringer solution. No damage of the mesothelial microvilli in the test specimens was found by transmission electron microscopy. After gentle blotting of the pleural surface for 1-2 min with filter paper, μ increased ~ 8 times; only a small part of this increase was due to liquid removal, because, if after blotting the mesothelium was wetted with Ringer solution, μ was ~ 6 times greater than that at control. Taking into account what reported in the above paragraphs, this finding suggests that blotting removes part of the mucopolysaccharides (and phospholipids) from the mesothelial coat, and damages or removes the microvilli.

In this research, we measured μ of the pleural mesothelium with the technique previously described (D'Angelo et al., 2004) in order to determine whether the postblotting addition of sialomucin, or hyaluronan, or both, lowered its value more than the addition of Ringer solution only, and whether this effect was such that μ approached its preblotting value. Moreover, we tried to test the effect on μ of postblotting addition of surfactant phospholipids, though the formation of

lamellar layers requires hours (Hills, 1989; 1992). Finally, we examined by transmission electron microscopy the microvilli of pleural mesothelium specimens before and after blotting.

2. Methods

Rib cage, lung, and diaphragm were obtained from 58 rabbits (2.6 – 3.5 kg b.w.) purchased from "G. Bettinardi", Momo (Novara). Animal experimentation was authorized by the Ministry of Health by decree N. 60/03A issued according to Order of the Excutive 116/92, in compliance with Directive 86/609/EC. Rabbits were anesthetized with an intravenous injection of 2 ml/kg of a mixture of pentobarbital sodium (Sigma, 12 mg/ml) and urethane (Sigma, 150 mg/ml). They were then heparinised (0.1 mg/kg), and killed by exsanguination. After removal of the skin and superficial muscles, the antero-lateral sides of the rib cage, the lungs (with closed trachea), and the diaphragm were removed. They were kept at ambient temperature (20 – 23 °C) in Ringer – bicarbonate solution (in mM: Na^+ 139, K^+ 5, Ca^{2+} 1.25, Mg^{2+} 0.75, Cl^- 119, HCO_3^- 29, D-glucose 5.6) through which 95% O_2 and 5% CO_2 was continuously bubbled. Up to 6 couples of specimen from each rabbit were used to measure the coefficient of kinetic friction (μ): no systematic difference occurred between the first and the last test, which was performed ~ 3-4 h later.

The apparatus used to measure frictional forces was that previously described (D'Angelo et al., 2004). It consists of a sliding platform connected through unextensible threads to the core of a differential transformer (Lynearsyn Sanborn 565 DT), and a balance arm held stationary at its fulcrum by a force transducer. Tissues specimens to be tested were fixed to the sliding platform, which was driven sinusoidally over a distance of 1 cm by an electric motor, and to a plexiglas rod attached to one end of the balance arm, which could rotate to maintain contact between the sliding and stationary tissues. The balance arm was held horizontal, and counterweights added to its other end enabled to change the normal force applied to the tissue from ~ 0.5 to ~ 8 g. Because the cross section of the rod was 0.62 cm^2, the pressure acting on the mesothelium ranged from ~ 0.8 to ~ 12.9 cmH_2O. The frictional force on the direction of motion was measured by the force transducer. The specimen of the rib cage, with the pleural surface facing upwards, was fixed to the sliding platform by a peripheral frame that was screwed down. Alternatively, the specimen of the diaphragm (mostly muscular), with the pleural surface facing

upwards, was pinned to a flat cork fixed on top of the platform. The specimen of the lung ~ 2 mm thick, with the pleura facing downwards, was held over the end of the rod with an O-ring. Alternatively, the specimen of diaphragm (tendinous or muscular), with the pleural surface facing downwards, was mounted on the rod. The tests were performed at a frequency of 0.28 Hz, corresponding to a peak sliding velocity of 0.88 cm/s; some tests were repeated at 0.96 Hz, corresponding to a peak velocity of 3.02 cm/s. Measurements of μ were made under the following sequence of conditions for each couple of specimens. 1) Ringer solution between specimens; 2) after application of filter paper on the mesothelium (see below); 3) Ringer solution; 4) five min after having placed sialomucin or hyaluronan solution between the mesothelial surface (see below); 5) Ringer solution after having washed out previous solution. We tried to make uniform the blotting of the mesothelial surface with the following procedure. After the initial measurement of μ with Ringer solution, the rod of the balance arm was raised, and if a liquid drop occurred on the surface of its specimen it was removed with the border of filter paper. Then a piece of filter paper was applied to the specimen on the platform, and a piece of plastic (PVC) with a smooth surface, an oval cross section of 3.2 cm^2, and a weight of 21.1 g was placed on the filter paper. The pressure on the mesothelium of platform specimen was, therefore, 6.6 cmH_2O, i.e. ~ half the maximum pressure applied to the mesothelial surfaces during their sliding for μ measurements (see above). After 1 min the plastic piece was removed, and the filter paper substituted if markedly wet. Then the rod was lowered so that now both specimens contacted the filter paper. Two counterweights were applied to the other side of the balance arm so that the pressure on the mesothelial surface was 5.5 cmH_2O. After 1 min the filter paper was removed, and the measurement of μ was performed. The sialomucin used was Sigma M3895(9-17% sialic acid); the molecular weight (m.w.) of this mucin has not been determined by the manufacturer. The m.w. of mucins ranges from 4 $x10^5$ Da (Tettamanti and Pigman, 1968) to 4 $x10^6$ Da (Bettelheim et al., 1962). The solubility of sialomucin in Ringer bicarbonate solution decreases above 25 mg/ml. Preliminary experiments were made to determine the

concentration of sialomucin to be used. We tested sialomucin solutions from 5 to 25 mg/ml, and found that these lowered the value of μ of post-blotting Ringer, and the effect increased with the concentration. Hence, we used a concentration of 25 mg/ml. As far as hyaluronan is concerned, that used by us (Sigma 53747, m.w. 1.63 x 10^6 Da) forms gel particles when its concentration in Ringer solution is greater than 3 mg/ml. Hence, we used a concentration of 2.5 mg/ml. Experiments were also performed with a mixture of sialomucin (25 mg/ml) and hyaluronan (2.5 mg/ml) in Ringer solution or with half these concentrations.

Though it is not known whether surface active phospholipids occur on the surface of mucopolysaccharide coat entrapped among mesothelial microvilli, we also tried to test the effect of phospholipids after mesothelial blotting. The phospholipids used (3 mg/ml) were dipalmitoylphosphatidylcholine 49%, dipalmitoylphosphatidylethanolamine 32%, and sphingomyelin 18% (Sigma, P0763, P1348, and S7004, respectively) according to Hills (1982). Because the dispersed phospholipids require hours to form lamellar layers (Hills, 1989; 1992), the above suspension was left 2 and ½ h in contact with the pleural specimens on which μ measurement was then performed. This time may be too short for lamellar layers formation, but a longer time may be detrimental for the specimen. A specimen of muscular diaphragm, pinned on a flat cork with the pleural surface facing upwards, was kept in a container immersed in Ringer solution (bubbled with O_2 and CO_2) up to a level that was just enough for its liquid surface to contact the upper border of the cork. The specimen was then covered by the above suspension, and the piston with a lung specimen was placed on it by mean of an adequate device. The container and the specimens were then covered by a plastic sheet. After 2 and ½ h the piston with its lung specimen was brought back to the balance arm, while the cork with its diaphragm specimen was fixed to the sliding platform, and μ measurements were performed.

Several specimens of visceral and parietal pleura were prepared for transmission electron microscopy. A few specimens were taken after μ measurements in initial Ringer to check whether the microvilli were preserved, as previously shown (D'Angelo et al., 2004). Most specimens were

taken after mesothelial blotting with filter paper, and μ measurements. The specimens were pinned on a cork and immersed in 3% glutaraldehyde in 0.12M phosphate buffer (pH 7.4) for 15 min. The specimens were then cut into small blocks which were immersed in the same fixative solution for 2 h and then postfixed in 1% OsO_4 in 0.18M phosphate buffer. The blocks were dehydrated in ethanol and embedded in araldite. Semithin sections were prepared for preliminary light microscopy. Ultrathin sections were stained with 3% alcoholic uranyl acetate and lead citrate, examined under the electron microscope (Jeol Jem 1010) and photographed at 8,000×.

Linear regressions between frictional force and load were computed with the least squares method and statistical assessment was made by covariance analysis. The results are presented as mean ± S.E. Statistical significance of group mean values was assessed by analysis of variance. The level of significance was taken at $P \leq 0.05$.

3. Results

Fig. 1 shows an example of the relationship between frictional force and load during oscillatory sliding of a specimen of intercostal pleura against one of visceral pleura at a peak velocity of 0.9 cm/s under the following sequence of conditions. 1) Ringer solution on the mesothelium, 2) after having blotted the mesothelial surface with filter paper (see Methods), 3) Ringer solution on the mesothelium, 4) sialomucin on the mesothelium (25 mg/ml in Ringer solution, see Methods), 5) Ringer solution on the mesothelium, after washout of previous solution. The mean values ± S.E. of the coefficient of kinetic friction (μ) from 14 tests obtained under the above conditions are reported in Table 1. With the initial Ringer solution (column 1) μ was similar to that previously found (D'Angelo et al., 2004). After having blotted the mesothelial surface with filter paper (column 2) μ increased ~ 5 times, somewhat less than previously found (8 times, D'Angelo et al., 2004), probably because of the more gentle blotting made in this research (see Methods). Ringer solution after blotting (column 3) decreased μ by 42%, i.e. more than previously found (21%, D'Angelo et al., 2004), but it was still ~ 3 times greater than that with the initial Ringer solution. Sialomucin solution (column 4) more than halved the value of μ obtained with post-blotting Ringer; consequently, μ with sialomucin solution was no longer significantly greater than that occurring with the initial Ringer solution. The value of μ with sialomucin solution did not change when the sliding velocity was increased to 3 cm/s (data not shown). After having washed out the solution with sialomucin, and replaced it with Ringer solution (column 5), μ increased again, and became significantly greater than that with sialomucin solution, but was still significantly lower than that with post-blotting Ringer. As shown in Fig. 1, after mesothelial blotting the intercept of the relationship at zero load was greater than that under the other conditions. This is because of the smaller radius of curvature of the air-liquid meniscus between the mesothelial surface, which adds a pressure because of surface forces (D'Angelo et al., 2004).

The mean values ± S.E. of μ under the sequence of conditions in which the effect of hyaluronan (2.5 mg/ml in Ringer solution, see Methods) was tested are reported in Table 2. With the initial Ringer solution μ was similar to that of Table 1; after having blotted the mesothelium with filter paper it increased 5.5 times. Ringer solution after blotting decreased μ by 57%, but it was still more than twice than with the initial Ringer solution. Hyaluronan solution nearly halved the value of μ obtained with post-blotting Ringer; consequently, μ with hyaluronan solution was no longer significantly greater than that occurring with initial Ringer solution. The value of μ with hyaluronan solution did not change when the sliding velocity was increased to 3 cm/s. After having washed out the solution with hyaluronan, and replaced it with Ringer solution, μ increased again, and became significantly greater than that with hyaluronan solution, but still significantly lower than that with post-blotting Ringer.

The mean values ± S.E. of μ under the sequence of conditions in which the effect of sialomucin (25 mg/ml) plus hyaluronan (2.5 mg/ml) was tested are reported in Table 3. The results are similar to those obtained with sialomucin or hyaluronan alone. The mean values ± S.E. of μ under the sequence of conditions in which the effect of sialomucin plus hyaluronan at half the above concentration was tested are reported in Table 4. The results are similar to those obtained with the higher concentration, though the effect on postblotting μ of sialomucin alone at 10-15 mg/ml was lower than that at 25 mg/ml (see Methods).

The mean values ± S.E. of μ under the sequence of conditions in which the effect of phospholipids (3 mg/ml in Ringer solution, see Methods) was tested are reported in Table 5. With the initial Ringer solution μ was similar to that of the other tests (Tables 1, 2, 3, and 4); after having blotted the mesothelium with filter paper it increased 6.6 times, i.e. within the range of the other tests (Tables 1-4). Ringer solution after blotting decreased μ by 53%, i.e. within the range of the other tests (Tables 1-4). Phospholipids suspension decreased μ by only 33% the value of μ obtained with post-blotting Ringer; consequently, at variance with the other tests, μ with phospholipids was significantly greater than that occurring with the initial Ringer solution.

After having washed out phospholipid suspension, and replaced it with Ringer solution, μ increased again, and became significantly greater than that with phospholipids.

Transmission electron micrographs of specimens in which μ measurements were performed after mesothelial blotting with filter paper showed a negligible, partial, or complete removal of the microvilli (Figs 2. and 3). This removal varied markedly from site to site of the same specimen.

4. Discussion

The measurements of the coefficient of kinetic friction (μ) of the pleural mesothelium show that the addition of sialomucin or hyaluronan in Ringer solution after mesothelial blotting with filter paper lowers μ more than the addition of Ringer solution only (Tables 1 and 2). This effect is such that after the addition of these macromolecules μ is no longer significantly higher than its preblotting value. The postblotting decrease in μ produced by Ringer is likely due to the hydration of the mesothelial coat. The decrease in μ produced by the addition of macromolecule solution is likely due part to the effects Ringer only, and part to the effect of sialomucin and hyaluronan, which improve boundary lubrication, though likely these macromolecules are not organized and connected as under physiological conditions. Indeed, the addition of Ringer solution, after washout of that of sialomucin or hyaluronan, increases the value of μ, in line with the dilution of the added macromolecules. These findings support the hypothesis that mesothelial blotting removes part of the macromolecules from the coat entrapped among the microvilli, and that these macromolecules are relevant for boundary lubrication of mesothelium. Indeed, D'Angelo et al. (2004) showed that their measurements of μ in pleural specimens sliding within a physiological range of velocities and load occurred under conditions of boundary lubrication. The same is the case for our measurements, even when sialomucin or hyaluronan were used, because μ did not change with sliding velocity, despite the marked increase in viscosity of the solution between specimens due to these macromolecules.

The transmission electron micrographs show that mesothelial blotting with filter paper removes and damages to various extent the microvilli (Figs. 2 and 3). The removal of microvilli implies a removal of part of the coat of macromolecules of the mesothelial surface described by Andrews and Porter (1973), because the polyanionic strands of this coat radiate from the microvilli and are interconnected with each other. On the other hand, some macromolecules may be removed from the surface of the coat even without removal of the microvilli because they may stick to the

filter paper during blotting. As a consequence, the few, not connected to the microvilli, are easily removed, while some of the others are detached from the microvilli, because these have been damaged by blotting. In any case, the mesothelial surfaces after blotting are different from those in initial Ringer. These morphological findings, therefore, fit in with the marked increase in μ produced by mesothelial blotting, and the fact that the addition of Ringer solution, though decreasing μ, fails to restore its preblotting value (D'Angelo et al., 2004; and present results). The partial or large removal of microvilli produced by mesothelial blotting makes even more remarkable the finding that postblotting addition of sialomucin or hyaluronan is able to restore μ to its preblotting value. This occurred also in the cases with the highest values of postblotting μ (0.2 - 0.3). Despite our attempt to standardize the procedure of mesothelial blotting with filter paper, both the increase in μ and the removal of microvilli produced by blotting were rather variable. This scattering, therefore, seems mainly due to different features of the specimens. Because most of the added macromolecules should not be bound to the rest of the coat and microvilli, it seems likely that the added macromolecules are washed out more easily than those physiologically occurring in the specimen. Indeed, if, after having measured μ with the initial Ringer, the specimens were washed out with other Ringer, μ did not increase appreciably. In this connection one has to consider that pleura specimens were kept in Ringer bubbled with O_2 and CO_2 (see Methods), and, therefore, they are in a condition close to, but not necessarily equal to the physiological one.

The lubricant relevance of hyaluronan between sliding tissues is known for a long time (Linn, 1967, Fraser and Laurent, 1996; Schmidt et al., 2007); by contrast, little information seems available on the lubricant proprieties of sialomucin. Sialomucins are cell-membrane associated mucins: they have been found on joint cartilage, where they showed lubricant relevance (Linn, 1968); moreover, they have been reported on mesothelial surface (Wang, 1985; Hilkens, 1992; Ohtsuka et al., 1997). At the concentration used (2.5 mg/ml) added hyaluronan should replace an appreciable amount of that removed by blotting, but should not attain the functional organization

likely occurring in the mesothelial surface before blotting. On the other hand, at the concentration used the molecules of hyaluronan form a nearly continuous network in the solution (Laurent, 1995), and this feature might be relevant. The morphological researches of Wang (1974, 1985) and Ohtsuka et al. (1997) show that in the coat covering the mesothelial surface the amount of sialomucin is much greater than that of hyaluronan. Our experiments show that the marked decrease in μ obtained by the postblotting addition of a solution with one of these macromolecules requires a concentration of sialomucin 10 times greater than that of hyaluronan. Moreover, our experiments show that the simultaneous addition of these macromolecules (table 3) produces results similar to those obtained by separate addition of each of them (Tables 1 and 2), as if a saturation were reached. On the other hand, similar results are also obtained by simultaneous addition of these macromolecules at half concentration. This indicates that, at these concentrations, the effect of hyaluronan is equivalent to that of a 10 times greater concentration of sialomucin, because the postblotting effect of sialomucin alone at 10-15 mg/ml was lower than that at 25 mg/ml (see Methods). The reverse should also be the case because it has been shown in the articular cartilage that the addition of a solution with 1 mg/ml of hyaluronan lowers μ less than one with 3 mg/ml (Schmidt et al., 2007).

Hills (1989; 1992) showed that surface active phospholipids placed on quartz plates are good boundary lubricant, particularly when several lamellar layers occur. On the other hand, the spatial and functional relationship between surfactant phospholipids and mucopolisaccharides on the mesothelial surface under physiological condition are not clear (see Introduction). Surfactant phospholipids are hydrophobic (Hills, 1989;1992): therefore, it is not like that under physiological conditions they occur on the surface of the mesothelial coat, which is in contact with pleural liquid water. If the surfactant phospholipids were free to move in the mesothelial coat, they would move to the surface of the specimen, where there is an air-liquid interface, but this is not necessary the case under physiological condition because of the lack of air in the pleural space. Moreover, surfactant phospholipids may interact with hyaluronan (Hills, 1989; Pasquali-Ronchetti et al.,

1997), and this could occur both under physiological conditions and in the pleural specimens. The experimental approach with the specimens, however, seems difficult because phospholipids require hours to form lamellar layers (Hills, 1989; 1992). Anyway, we tried to test phospholipids. The addition of surfactant phospholipids after mesothelial blotting (Table 5) did not lower μ so much as the addition of sialomucin or hyaluronan. On the other hand, this finding must be taken cautiously because the contact time of phospholipids with the specimen (2 and ½ h) before μ measurements might have been too short for lamellar layers formation (see Methods).

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Legends

Fig. 1. Relationship between frictional force and load during oscillatory sliding of a specimen of intercostal pleura against one of lung pleura under condition sequence reported on inset.

Fig. 2. Transmission electron micrographs of two specimens of visceral pleura after mesothelial blotting and μ measurement. Microvilli are completely (A) or partially (B) removed. Bars = 1 μm.

Fig. 3. Transmission electron micrographs of a specimen of visceral (A) and one of parietal (B) pleura after mesothelial blotting and μ measurement. Negligible (A) and complete (B) removal of microvilli. Bars = 1 μm.

Figure

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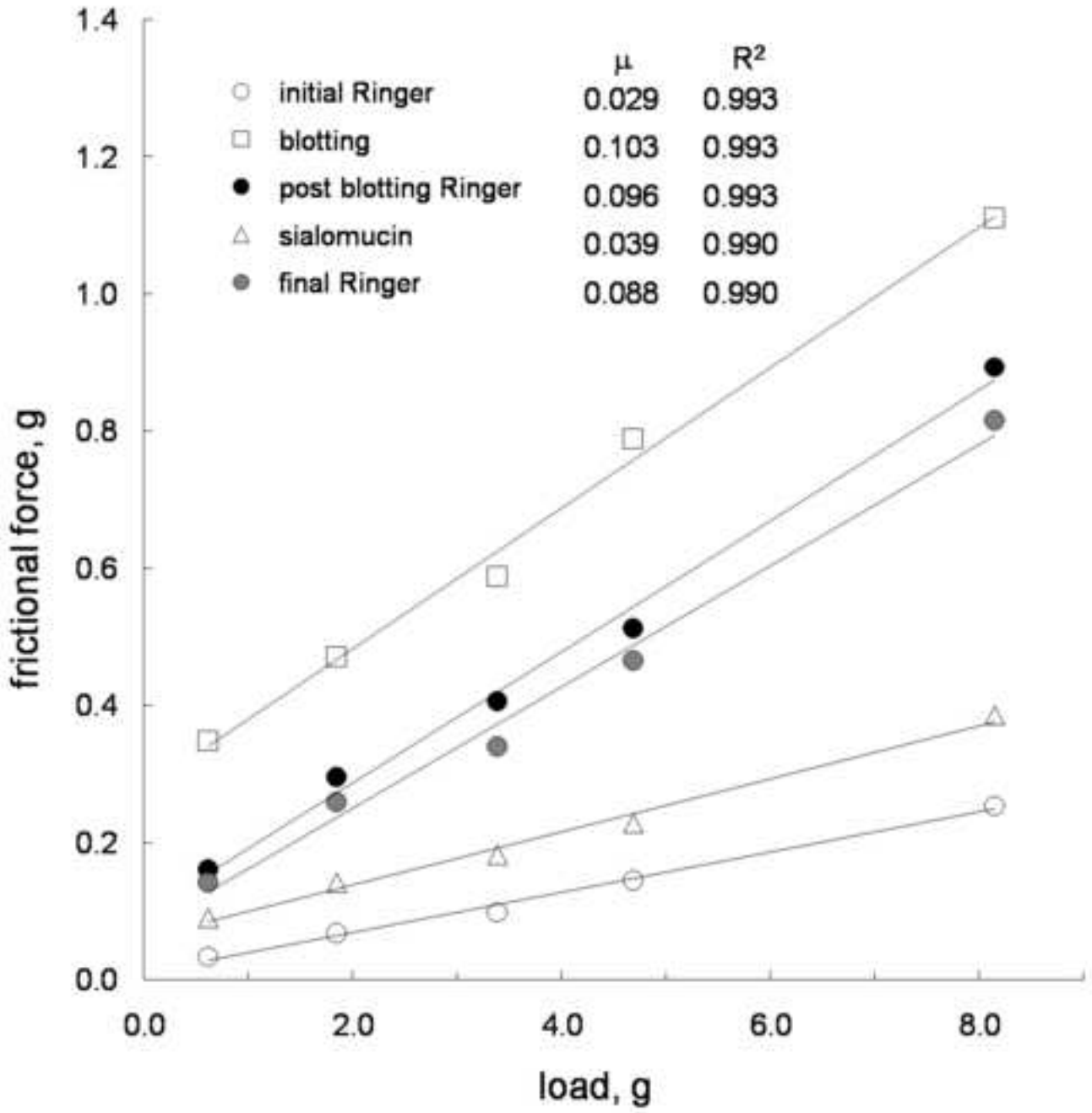

Figure2

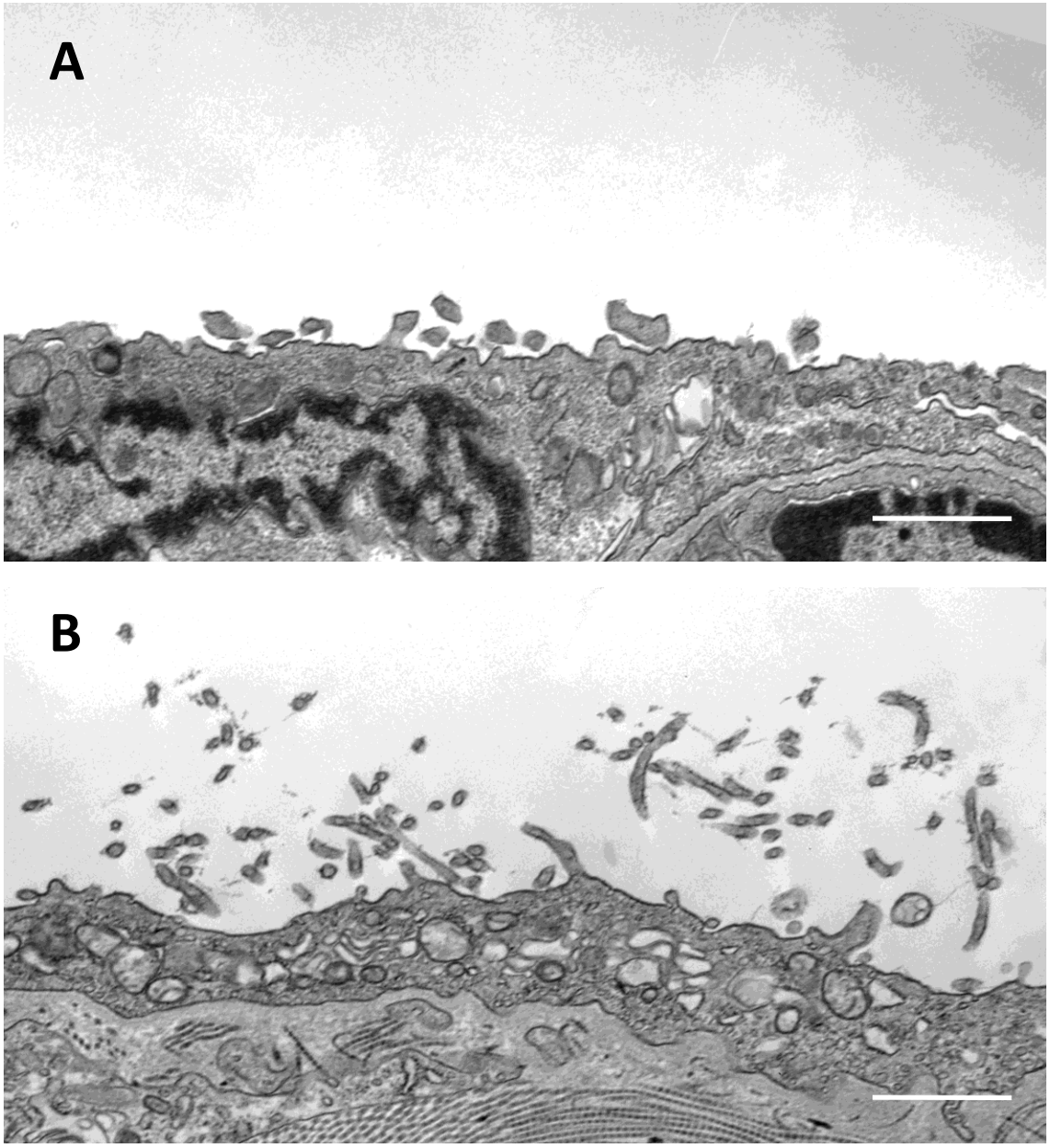

Figure3

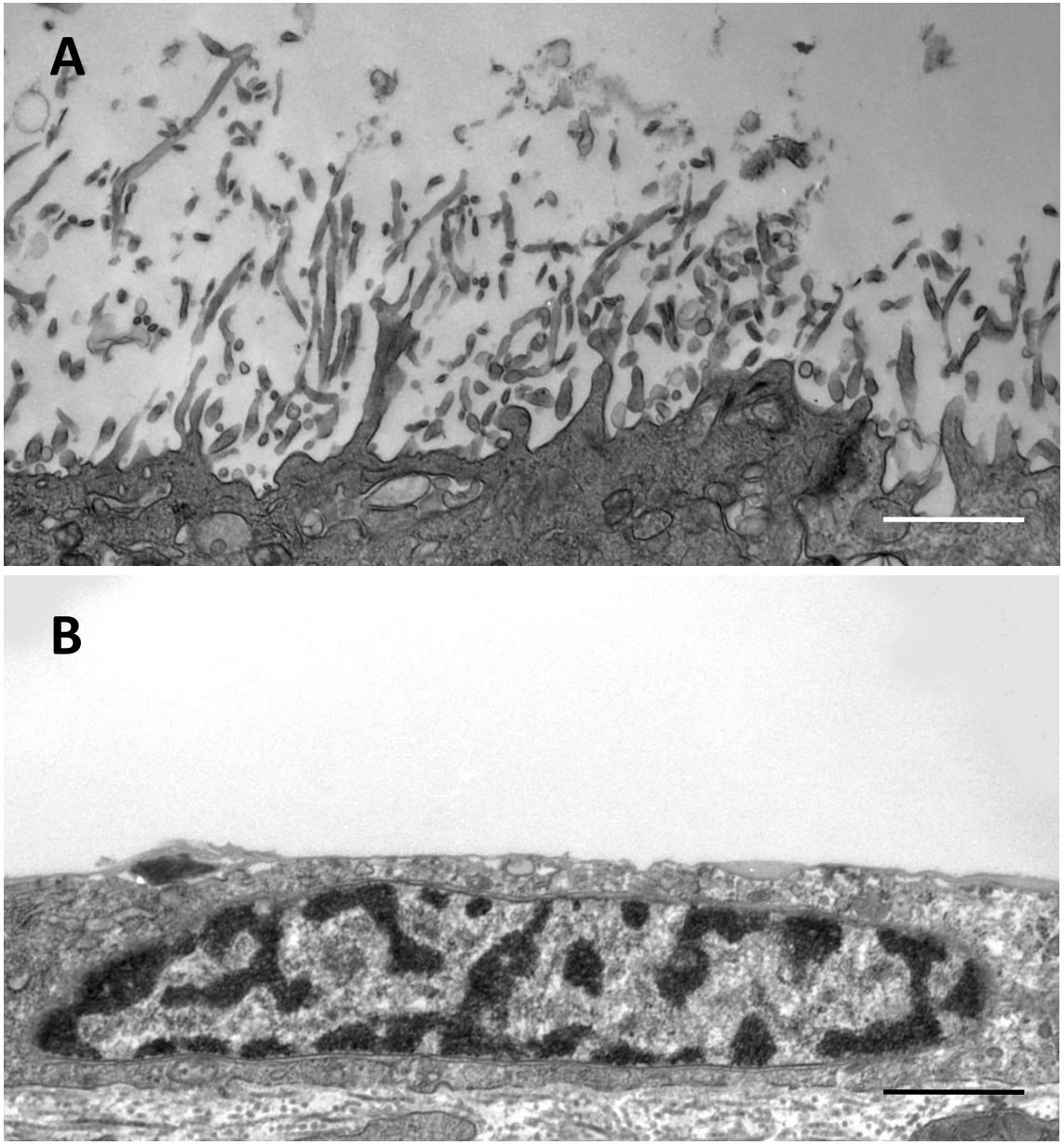

Table 1

Table 1

Effect of sialomucin (25mg/ml) on coefficient of kinetic friction (μ) of pleural mesothelium after blotting with filter paper, and rewetting with Ringer solution

	1	2	3	4	5					
Condition	Initial Ringer	Blotting	Post blotting Ringer	Sialomucin	Final Ringer	Δ 2-3	Δ 3-1	Δ 3-4	Δ 4-1	Δ 5-4
μ	0.028	0.143	0.083	0.036	0.063	0.060	0.055	0.047	0.008	0.028
± SE	0.003	0.014	0.009	0.003	0.007	0.015	0.009	0.007	0.004	0.005
t						3.95	6.00	6.95	2.09	4.53
P						<0.01	<0.01	<0.01	>0.05	<0.01

Values are means ± S.E.; N = 14 (8 lung – intercostal, 4 lung – diaphragm, and 2 diaphragm –intercostal).

Table 2

Table 2

Effect of hyaluronan (2.5mg/ml) on coefficient of kinetic friction (μ) of pleural mesothelium after blotting with filter paper, and rewetting with Ringer solution

	1	2	3	4	5					
Condition	Initial Ringer	Blotting	Post blotting Ringer	Hyaluronan	Final Ringer	Δ 2-3	Δ 3-1	Δ 3-4	Δ 4-1	Δ 5-4
μ	0.029	0.161	0.068	0.035	0.049	0.092	0.039	0.033	0.006	0.014
± SE	0.002	0.017	0.010	0.005	0.008	0.018	0.010	0.006	0.005	0.003
t						5.27	3.77	5.27	1.22	4.23
P						<0.01	<0.01	<0.01	>0.05	<0.01

Values are means ± S.E.; N = 14 (9 lung – diaphragm, 4 lung – intercostal, and 1 diaphragm – intercostal).

Table 3

Table 3

Effect of sialomucin (25 mg/ml) plus hyaluronan (2.5mg/ml) on coefficient of kinetic friction (*μ)* of pleural mesothelium after blotting with filter paper, and rewetting with Ringer solution

	1	2	3	4	5					
Condition	Initial Ringer	Blotting	Post blotting Ringer	Sialomucin+ Hyaluronan	Final Ringer	Δ 2-3	Δ 3-1	Δ 3-4	Δ 4-1	Δ 5-4
μ	0.024	0.180	0.082	0.029	0.058	0.098	0.058	0.053	0.005	0.025
± SE	0.003	0.032	0.007	0.002	0.006	0.027	0.006	0.006	0.003	0.004
t						3.69	9.13	9.27	1.89	6.63
P						<0.01	<0.01	<0.01	>0.05	<0.01

Values are means ± S.E.; N = 12 (9 lung – intercostal, 3 lung – diaphragm).

Table 4

Table 4

Effect of sialomucin (12.5 mg/ml) plus hyaluronan (1.25 mg/ml) on coefficient of kinetic friction (μ) of pleural mesothelium after blotting with filter paper, and rewetting with Ringer solution

	1	2	3	4	5					
Condition	Initial Ringer	Blotting	Post blotting Ringer	Sialomucin+ Hyaluronan	Final Ringer	Δ 2-3	Δ 3-1	Δ 3-4	Δ 4-1	Δ 5-4
μ	0.026	0.199	0.072	0.031	0.052	0.126	0.047	0.041	0.005	0.021
± SE	0.002	0.021	0.010	0.004	0.011	0.020	0.010	0.007	0.004	0.004
t						6.27	4.71	6.12	1.16	4.98
P						<0.01	<0.01	<0.01	>0.05	<0.01

Values are means ± S.E.; N = 10 (8 lung – diaphragm, 2 lung – intercostal).

Table 5

Table 5

Effect of phospholipids (3mg/ml) on coefficient of kinetic friction (μ) of pleural mesothelium after blotting with filter paper, and rewetting with Ringer solution

	1	2	3	4	5					
Condition	Initial Ringer	Blotting	Post blotting Ringer	Phospholipids	Final Ringer	Δ 2-3	Δ 3-1	Δ 3-4	Δ 4-1	Δ 5-4
μ	0.029	0.198	0.094	0.063	0.078	0.104	0.064	0.031	0.033	0.016
± SE	0.004	0.029	0.015	0.012	0.015	0.027	0.013	0.008	0.010	0.004
t						3.87	4.79	4.12	3.17	3.50
P						<0.01	<0.01	<0.01	<0.02	=0.01

Values are means ± S.E.; N = 8 (all lung – diaphragm).