

## SURFACE-ACTIVE PHOSPHOLIPID IN MUSCLE LYMPH AND ITS LUBRICATING AND ADHESIVE PROPERTIES

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### ABSTRACT

*In order to investigate the role of phospholipid in facilitating lymph flow, "deep-thigh" lymph was collected from ten anesthetized dogs and analyzed for phospholipids using thin-layer chromatography. The lymph was found to be surface active at liquid and at solid surfaces at which it deposited a hydrophobic monolayer in vitro. Extracted phospholipid was found to be an effective release agent as a monolayer, reducing the force of adhesion of 5% protein solutions by 76% according to a standard test for tacky glues. The same monolayers were effective lubricants, reducing friction by 96%; while mixtures of the same phospholipids from synthetic sources gave similar results for release and lubrication. Surfaces in contact with extracellular fluid or lymph in vivo were found to be hydrophobic with a drop of saline on semitendinosus muscle fibers displaying a contact angle of  $40.2^{\circ} \pm 7.2^{\circ}$ . The results are considered compatible with the hypothesis that surface-active phospholipid facilitates the flow of lymph; while it could also provide boundary lubrication for sliding of connective tissue in locomotion and for any relative movement of motor units in muscle contraction and fatigue.*

An adequate flow of lymph is necessary in order to prevent edema and maintain the colloid osmotic pressure needed for homeostasis according to the Starling Hypothesis (1). However, lymph derived from most tissues can contain up to 5-8%

protein (2)--a concentration at which such solutions can gel at room temperature.

Solutions of albumins and globulins are not only strong adhesives but demonstrate "tack." Tack is the instant adhesion sometimes experienced as the stickiness felt upon spilling lymph or serum on one's fingers.

These simple physical properties raise the question of how the lymphatic system can remain functional especially when any sticking of valve cusps at an inappropriate phase of the lymphatic pumping cycle could severely compromise flow. Lymph vessels are very flaccid structures with wall-to-wall contact and contact between cusps (3). The presence of tacky and potentially potent adhesives between such touching or near-touching walls has led (4) to the question: why does the whole lymphatic system not get "gummed up?" One approach to this question is provided by the observation that, in several other regions of the body where proteins occur naturally between touching surfaces, surface-active phospholipid is also found. These sites include the Eustachian tube (5), the pleura (6), and the gastric mucosa (7,8). These surfactants, and especially their mixture in the middle ear, have been found (5) to be very effective "release agents" or "adhesives" as they are often termed in the physical sciences. Moreover, they have been shown to be effective against adhesion by proteins either when adsorbed to the potentially adhering surfaces or when compounded into the bulk of the adhesive itself (4).

Lymph also provides superb lubrication of tendon and other connective tissue essential for efficient locomotion, so tests have been included to see whether the entrained phospholipids lubricate the muscle and tendons of the thigh as indicated by studies of tissues such as pleura (6), pericardium (9) and articular cartilage (7,10).

Lymph was collected from the thigh for surface activity and subjected to phospholipid analysis. Extracted phospholipid was then tested for its adhesive and lubricant properties (4,9). Tests for adsorption consisted of measuring the hydrophobicity of surfaces exposed to extracellular fluid and lymph *in vivo* and any reduction when those surfaces are rinsed with solvents for phospholipids. This is based upon the premise that all natural membranes should be very hydrophilic by virtue of the orientation of protein and phospholipid molecules according to standard theory for their structure based upon lipid bilayers (11,12).

## MATERIALS AND METHODS

### *Lymphatic cannulation*

Mongrel dogs (15-25kg) of either sex were anesthetized with pentobarbital sodium (30mg/kg i.p.) and intubated to permit spontaneous respiration with forced inflation every 15 minutes to prevent atelectasis. Cannulation of a deep lymphatic in the thigh followed the procedure described in detail by Jacobsson and Kjellmer (13). Lymphatics were visualized by subcutaneous injection of Patent Blue Violet (11%), and all were ligated except one large deep vessel which was cannulated with a polyethylene catheter (PE-10).

### *Lipid extraction procedure*

Samples of lymph were transferred from glass collection bottles to Teflon-capped tubes for solvent extraction by the method of Folch et al. (14,15) using a 2:1 chloroform:methanol mixture. A contact angle was measured on the inside of the empty glass bottle using a goniometer as

described below and the test repeated after rinsing with the same solvent and adding the rinsings to the solvent extract (20ml) from the lymph sample itself. After agitating in a Vortex for 1 minute, 4ml of 0.2N KCl in methanol were added. After agitating for another minute, the tubes were centrifuged at 3,500rpm for 15 minutes at 18°C and the contents allowed to settle when the lower chloroform phase was separated and evaporated to dryness under nitrogen. The extracted phospholipid was weighed and stored at 0°C.

### *Thin-layer chromatography (TLC)*

Quantitative analysis of the phospholipids from each sample of lymph was carried out using two-dimensional TLC. Fifty microliters of each extract were "spotted" on precoated silica gel plates (Silica Gel H, Analtech). Spot separation was implemented on 20x20cm glass plates in solvent-saturated chambers. The solvent system for the first phase consisted of chloroform:methanol:acetic acid:water (50:25:8:4), and the second phase consisted of the same solvents in a ratio of 50:7:5:8:2. The separated phospholipid spots were developed using a 97:3 spray of concentrated sulphuric acid and formaldehyde (37%). The plates were then heated to 180°C for 20 minutes to yield blackened spots suitable for identification.

Synthetic phospholipids (Sigma), serving as controls, were spotted parallel to the rise of each phase. After plate development, the spots were transcribed onto graphic tracing paper for determination of their  $R_f$  factors and for permanent record.

### *Phosphorus determination*

After identification, the isolated phospholipid spots were quantified according to their phosphorus content using the method of Rouser et al. (16). After plate development, the identified spots were circled and numbered together with several blank areas to serve as controls, i.e., silica blanks. The encircled spots

were scraped into extraction tubes and treated by adding 0.9ml of 70% perchloric acid (Suprapur, MCB), heating at 180°C for 20 minutes, cooling, and then mixing with ammonium molybdate (0.4ml of a 1.25% solution for small spots or 0.5ml of a 2.5% solution for large spots) for color reaction. Ascorbic acid was then added for reduction of the compound (0.4ml of a 5% solution for small spots or 0.5ml of a 10% solution for large spots). The samples were then centrifuged briefly before being measured for optical density (Gilford-260 UV-VIS spectrophotometer) at 797λ. Reagent blanks were used for optical density correction following a zero setting. The quantity of each phospholipid was thus determined as a weight of phosphorus.

#### Friction test

The method adopted (9) for assessing the ability of the surfactants to reduce friction between moving surfaces was based upon a standard method (17) which measures the force (F) needed to prevent movement of a yarn passing over a rotating pulley on which it continually slips (see Fig. 1). The coefficient of kinetic friction ( $\mu$ ) is then derived from the following equation (10):

$$F/W = e^{\mu\theta} \quad (1)$$

where  $\theta$  is the angle (in radians) by which the yarn is diverted when passing over the pulley and W is the counterbalancing weight. Typical values in these experiments were 55° for  $\theta$  and 6.68g for W to give F of the order of 130-200g for controls or 6-30gm for runs with lubricant. All friction tests were performed at 37°±1°C and relative humidity within the range 80-90%. A synchronous motor was used to avoid any dependence of the speed on frictional load.

The yarn used was cotton carboxylated according to the standard method with the exact carboxyl content then determined by another standard method as adapted by Hills (18). In these tests the carboxyl content was 5.99%, indicating

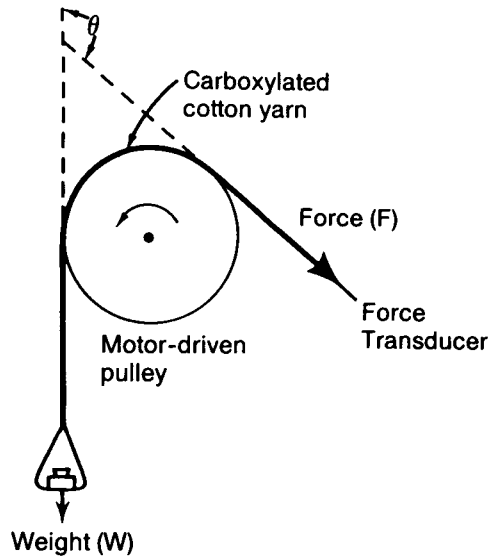


Fig. 1. Demonstrating the principle of a standard method (17) for measuring the coefficient of kinetic friction ( $\mu$ ) recommended by the British Standards Institute (1974). The yarn, with or without lubricant, passes over a rotating pulley on which it is continually slipping, the force (F) recorded on the force transducer being related to W,  $\theta$  and  $\mu$  by Equation 1.

the percentage of the hydroxyl groups that had been replaced by carboxyl groups. This was undertaken to simulate the situation that arises in all natural membranes where ionized carboxyl groups inherent in the structure (11) provide a prime site for the adsorption of cationic surfactants (7,18).

#### Surfactants

The surfactant was applied to the fiber by soaking the carboxylated cotton yarn in the phospholipid extract from each lymph sample and then allowing the chloroform to evaporate. Control samples were soaked in chloroform only. Extrinsic synthetic and purified surfactants were also tested. These included a commercial mixture of naturally occurring phosphatidylcholines (PC, Sigma P8640), sphingomyelin (Sp, Sigma S7004), a commercial mixture of naturally occurring phosphatidylethanolamines (PE, Sigma P9137), a commercial mixture of naturally occurring

phosphatidylinositols (PI, Sigma EP-5766), a commercial mixture of naturally occurring phosphatidylserines (PS, Sigma P8515), and a mixture of the above approximating their relative preponderance in lymph (see Table 1), except that the lysophosphatidylcholines were omitted. The mixture used was 20:9:3:1:1, PC:Sp:PE:PI:PS.

**Table 1**  
**Thin-Layer Chromatographic Analysis of Canine 'Deep Thigh' Lymph**

Phospholipid Group	% of Lymph
Phosphatidylcholine (PC)	57.2±3.3
Sphingomyelin (Sp)	22.1±1.7
Phosphatidylethanolamine (PE)	10.0±1.2
Lysophosphatidylcholine (LPC)	4.6±1.3
Phosphatidylserine (PS)	3.3±1.0
Phosphatidylinositol (PI)	2.7±0.9
Phosphatidic acid (PA)	Trace
Phosphatidylglycerol (PG)	Trace

Values are means ± S.E.M. for one sample from each of 10 dogs.

Each was made up to a concentration of 8mg/l in chloroform and the solutions were kept at 0°C under nitrogen. This concentration was selected to give an estimated surface concentration in the region of physiological values (6) after soaking the yarn in the solution and allowing the chloroform to evaporate.

### Monolayer deposition

The motor-driven pulley shown in Fig. 1 is a cylindrical glass tube (1.5cm dia.) onto which the same surfactant used to coat the yarn was deposited as an orientated monolayer according to standard procedures developed for flat surfaces by Blodgett (19) as updated by Gaines (20). The surfactant was deposited as 4ml of its solution in hexane (2.5µg/ml) onto the surface (150cm<sup>2</sup>) of a pool of saline at 44±0.5°C contained in a Langmuir trough fitted with a leak-proof Teflon Barrier--see Fig. 2. The glass cylinder was previously suspended with its axis vertical and partially immersed in the saline. After the solvent had evaporated, depositing a sur-

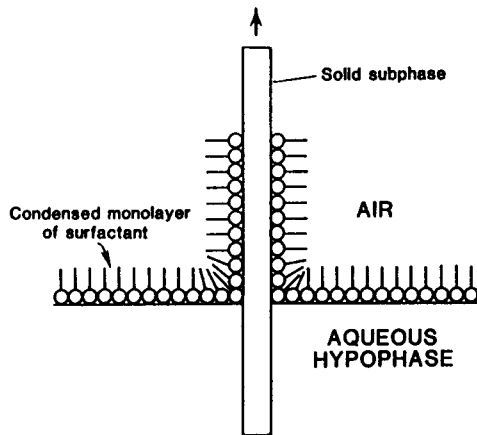


Fig. 2. Demonstrating the principle devised by Blodgett (19) whereby surfactant compressed into a condensed monolayer on the pool of a Langmuir trough can be transferred onto a glass surface by raising the plate. Note that the polar headgroups of the surfactant molecules are now orientated towards the glass, thus facilitating adsorption.

factant film, the barrier was advanced to reduce its area from 150 to 90cm<sup>2</sup> during which time the surface tension was monitored using a Wilhelmy balance with its platinum flag also dipping into the trough. The glass cylinder was then slowly withdrawn and the barrier advanced so as to maintain a constant reading on the Wilhelmy balance. Before repeating the procedure, all trough surfaces plus the next glass cylinder to be coated were cleaned with phosphate-free detergent, rinsed with distilled water, and soaked in concentrated sulphuric acid containing potassium dichromate (saturated) for at least 30 min. This was followed by rinsing with a large volume of distilled water and then drying in air. The same cleaning procedure was used on the controls.

Each clean glass cylinder was placed in the friction tester with an uncoated carboxylated yarn as shown in Fig. 1., and the force was measured three times using a force displacement transducer (Grass model 10D), stopping the pulley between each measurement. The control yarn and glass cylinders were then coated with surfactant or extract as described above and the test was repeated three times. The

whole procedure of six measurements was repeated three or four times starting each time with a different glass cylinder and carboxylated cotton fiber, with three pulleys and three yarns per sample for 12-18 runs in total. The foregoing was repeated for the phospholipid extract from each lymph extract, each synthetic, and the mixture of synthetics approximating the natural composition. In all cases, the cylindrical glass tube was treated with the identical composition of surfactant used to coat the fiber.

### Adhesive testing

The assessment of the ability of the extracts and their components to act as release agents was based on a standard method for testing adhesives devised by the American Society for Testing Materials (21)--ASTM test No. C908-79. The apparatus is shown in Fig. 3 and consists

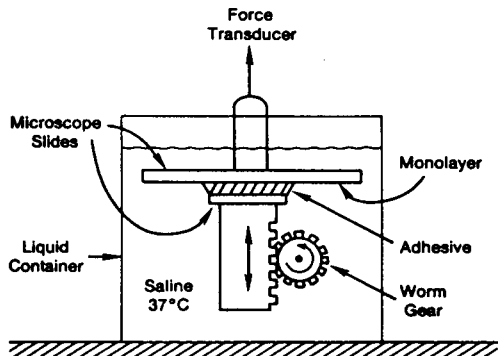


Fig. 3. A standard test recommended for tacky adhesives by the American Society for Testing Materials (21). The force is measured for separating two plates (microscope slides) sandwiching protein solution after they have been pressed together for a given time and pressure.

of two microscope slides placed horizontally with their axes perpendicular. Thus, they come into contact over an area 2.5x2.5cm. The lower plate is connected to a screw for lowering it while the upper is connected to a force-displacement transducer and pen recorder that records the maximum force generated in separating the two surfaces. Albumin was used as the adhesive since it is the predomi-

nant protein in serum and lymph (22). A weight of 500g is applied for 30 seconds and then the plates are pulled apart, recording the maximum force needed for separation. The weight of the upper plate is then subtracted to give the net adhesive force ( $F_1$ ). The plates are then rigorously cleaned, and the test repeated with the exception that one plate is now coated with a monomolecular layer of the phospholipid extract and the new force ( $F_2$ ) recorded. The quotient of  $(F_1 - F_2)/F_1$  is now taken as the ability of the coating to act as a release agent, ranging from 0 for no effect ( $F_1 = F_2$ ) to a value of 1 for perfect release, i.e., no adhesion ( $F_2 = 0$ ) remaining after coating one surface with a monolayer.

### Excised muscle

After sacrificing the dog with an overdose of pentobarbital, the semitendinosus muscle was excised and adhering fascia carefully dissected to expose a surface of muscle fibers. This surface was then well rinsed with saline and adhering water allowed to evaporate. During this period, the hydrophobicity of the rinsed surface was measured as the contact angle for a drop ( $5\mu\text{l}$ ) of saline using the standard procedure of Sherman (23). The contact angle is the angle between the tissue surface and the tangent to the air-liquid interface at the triple point where all three phases meet. It ranges from 0 for a perfectly wettable surface to values such as  $108^\circ$  for Teflon (24) and higher and is easily measured in the magnified ( $\times 25$ ) field of view of a goniometer fitted with cross-wires to align with the interfaces--Fig. 4. The plateau value of the contact angle was recorded in each case.

## RESULTS

### Lymphatic cannulation

The slipperiness of the lymph ducts in attempting to hold the cannula in place and of individual fiber bundles carefully

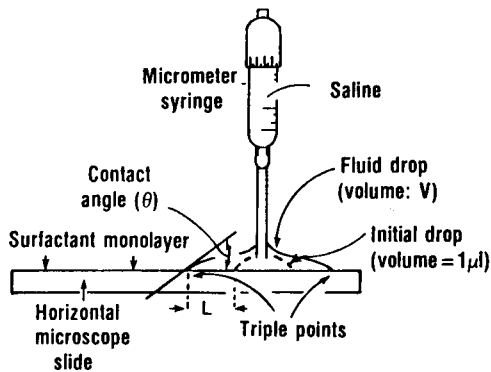


Fig. 4. Apparatus used for measuring contact angle imparted by a drop of saline applied to a monolayer of surfactant adsorbed to glass, the same method being used for tissue. The contact angle is measured by rotating crosswires in the magnified field of view of a goniometer until one coincides with the tissue surface and the other forms a tangent to the liquid-air interface at the triple point.

separated from the main bulk of the muscle gave the qualitative impression of the presence of a particularly effective lubricant. The same impression was obtained of adjacent connective tissue.

#### Phospholipid in lymph

Total phospholipid for 10 samples from as many dogs was  $107.5 \pm 15.9$  mg/100 ml. Results for the analyses of these samples by TLC are given in Table 1.

#### Surface activity

Each sample of lymph foamed readily upon shaking, indicating a low surface tension. Contact angles measured on the surfaces of the empty glass vials used to collect the lymph averaged  $47.6 \pm 2.4^\circ$  which was reduced to  $15.4 \pm 1.0^\circ$  after rinsing with the phospholipid solvent.

#### Lubrication

The coefficient of kinetic friction of yarn on glass was reduced from  $3.12 \pm 0.07$  to  $0.16 \pm 0.01$  ( $n=10$ ). This represents a reduction of 94.9%, reaching a highly significant level statistically ( $P < 0.001$ ) using the *t* test. A mixture of synthetic phospholipids of the same composition as listed in Table 1 gave  $\mu = 0.13 \pm 0.01$  ( $n=10$ ), corresponding to a reduction of 95.8%.

#### Adhesion

The release test was performed on monolayers derived from each of the 10 lymph samples from as many dogs. The mean force of separation ( $\pm$ s.e.m.) for control surfaces glued by 5% bovine albumin in saline was  $F_1 = 62.8 \pm 2.1$  gms ( $9.73$  gm.cm<sup>2</sup>). When the surfaces were previously coated with a monolayer of extracted phospholipid, this force was reduced to  $F_2 = 14.85 \pm 3.49$  gms ( $2.30$  gm.cm<sup>2</sup>). This represents a reduction,  $(F_1 - F_2)/F_1$ , of 76%, the reduction being statistically significant ( $P < 0.01$ ). Using the mixture of synthetic phospholipids of the composition given in Table 1,  $F_1 = 13.19 \pm 2.24$  gms ( $n=10$ ) corresponding to a release factor of 79%.

#### Ancillary experiments

It was noted that if a large quantity of phospholipids were applied to the surface by solvent deposition and evaporation, e.g. enough for 100 monolayers, then no significant adhesion occurred at all in 20 tests, i.e. release exceeded 99%. Similarly coefficients of friction could be obtained by the lubrication test which were an order of magnitude lower than those reported above, i.e. below 0.015 in all cases (20 tests).

#### Surface hydrophobicity

Contact angles for 10 exposed surfaces of excised semitendinosus muscle averaged  $40.2 \pm 7.2^\circ$  and were reduced to

27.4°±3.2° after solvent rinsing. This reduction reached the 99% significance level using the paired t test.

## DISCUSSION

There is no doubt from the analysis of deep-thigh lymph that it contains relatively large quantities of phospholipid (107mg/100ml). This value is in good agreement with estimates derived from organic phosphorus contents of canine cervical lymph of 5.9mg/100ml (2) when considering that most phospholipids have molecular weights in the region of 700-900 and the atomic weight of phosphorus is 15. The TLC analysis shows that the major component (57.1%) is phosphatidylcholine which, in its saturated form in the lung, is highly surface active (25). The overall analysis (Table 1) corresponds closely to that of lymph derived from the lung (26), where the composition indicates a vascular source for phospholipid and the same arguments would seem to apply to the material collected in these experiments. The overall phospholipid content of lymph collected in these studies (107mg/ml) is less than that of canine serum-211mg/100ml (22)--but roughly reflects the partition of protein between plasma and lymph (6.18% vs 3.32% according to Brobeck (2). Thus, protein could be acting as a carrier for phospholipid, presumably as lipoprotein (27), or it would be very difficult to see how a substance as insoluble as phosphatidylcholine could be transported in a non-membranous form (7). Proteins have also been attributed the ability to facilitate phospholipid transport across membranes (28)--a process necessary for the phospholipid in pericapillary filtrate and, hence, in lymph to be derived from plasma.

Having demonstrated the presence of phospholipid in muscle lymph in appreciable quantities consistent with a vascular source, it is interesting to note its effectiveness as a release agent. As an adsorbed monolayer it has the capability to reduce the adhesive power of albumin by 76% *in vitro*. This agrees well with a figure of 79% for pulmonary lymph ob-

tained by Hills et al (26). It would be difficult to attribute this result to a contaminant because a synthetic mixture of essentially the same phospholipids listed in Table 1 gave a very similar result (79% reduction). This does not mean that the phospholipid present will necessarily exert the same effect *in vivo*. However, the hydrophobic nature of muscle fiber surfaces and the similarity in contact angles between those measured on tissue and on glass are consistent with an adsorbed layer of phospholipid. Moreover, the contact angle is reduced by phospholipid solvents, although it is uncertain whether these solvents would also elutriate phospholipids from the underlying cellular membranes. On the other hand, gross histological observation by light microscopy showed no obvious evidence of this phenomenon.

The coefficient of kinetic friction ( $\mu$ ) of 0.13 agrees well with values of 0.15 for a similar mixture of phospholipids extracted from synovial fluid and 0.19 for the corresponding mixture of synthetics determined by Hills and Butler (10). These values demonstrate the capability of the phospholipid mixture in muscle lymph to provide good lubrication. Moreover, this is *boundary lubrication* as originally described by Hardy (29) for solid-to-solid contact where sliding is facilitated by any surfactant. This differs from fluid-film lubrication in the form of hydrodynamic or hydrostatic lubrication and would seem much more likely to occur with the low speeds involved in physiological movement than in engineering where those concepts were derived (30). Thus, the movement of tendon and other connective tissue essential for efficient limb movement could be facilitated by boundary lubrication provided by the surface-active phospholipids in the lymph derived from muscle. The same mechanism probably applies to other tissues. It is also likely that any sliding of motor units relative to each other during muscular contraction and fatigue would be lubricated by the same system.

The same phospholipid lining facilitating sliding is also likely to help in initi-

ating motion by preventing sticking in a shearing mode. It is well known in the physical sciences that industrial surfactants which are good release agents also tend to impart good "lubricity" (31).

A good release agent could facilitate lymph flow in several ways. Firstly, it could prevent sticking of valve surfaces vital to the lymphatic "pump" as it is commonly termed (32) and of surfaces of the flaccid lymph vessels where opposite walls touch or are sufficiently close for "tack" or the "setting" of protein solutions to compromise flow. One could envisage a partially "glued" lymph vessel where an increase in flow would aid in reversing the "setting" of protein solutions, thus reducing flow resistance. Hence, it is interesting to find that the relaxation period for the rheology of protein solutions of about one hour according to Kragh & Wootton (33) is of the same order as the time scale for resistance changes for lymph flow in the lung (34,35).

The second possible mode of action of surface-active phospholipid in facilitating flow is in modifying the viscosity of lymph just as its commercial form, "lecithin", can reduce the viscosity of chocolate by 60% (7) when added as only 0.1%, i.e., less than the concentration of 0.107% found in muscle lymph in this study. A third possible means of facilitating lymph flow could occur if surfactant were acting as a lubricant to facilitate flow by "slippage" at the wall--a mode most appropriate if there were any "setting" of the protein to give a degree of "plug flow." In its fully developed state, "plug flow" is observed when squeezing toothpaste out of a tube. However, its occurrence would require lower coefficients of friction than the values of 0.13 obtained in these studies of *monolayers*. On the other hand, the ancillary experiments indicated phenomenal release and lubrication when using much thicker coatings of phospholipid, i.e. using quantities capable of providing oligolamellar linings. Oligolamellar lubrication which was comparatively recently introduced into engineering has been put forward (7)

as the mode by which surface-active phospholipid in synovial fluid provides the phenomenal lubrication of the articular surfaces. However, no lamellated structures could be found in published electron micrographs of lymph vessels, although the reasons why the common fixatives would destroy such hydrophobic surface layers has been discussed in much detail elsewhere (7).

In conclusion, there is no doubt that phospholipid is present in appreciable quantities in muscle lymph and that it has the capability to act as an excellent lubricant and release agent *in vitro*, both properties being desirable in maintaining patency of the lymphatic system.

#### REFERENCES

1. Starling, EH: On the absorption of fluids from the connective tissue spaces. *J. Physiol.* 19 (1896), 312-326.
2. Brobeck, R: *Physiological Basis of Medical Practice*, 9th Ed., Williams & Wilkins, Baltimore (1973).
3. Casley-Smith, JR: Are the initial lymphatics normally pulled open by the anchoring filaments? *Lymphology* 13 (1980), 120-129.
4. Hills, BA: Arrest of metastatic cells: Agents promoting and inhibiting non-specific adhesion by fibronectin. *Anticancer Res.* 5 (1985), 287-292.
5. Hills, BA: Hydrophobic lining of the Eustachian tube imparted by surfactant. *Arch. Otolaryngol.* 110 (1984), 779-782.
6. Hills, BA, BD Butler, RE Barrow: Boundary lubrication imparted by pleural surfactants and their identification. *J. Appl. Physiol. (Respiration, Environmental Exercise Physiology)*, 53 (1982), 463-569.
7. Hills, BA: *The Biology of Surfactant*, Cambridge U.P., Cambridge (1988).
8. Hills, BA, BD Butler, LM Lichtenberger: Gastric mucosal barrier: Hydrophobic lining of the lumen of the stomach. *Am. J. Physiol. (Gastrointestinal and Liver Physiology)*, 7 (1983), G561-568.
9. Hills, BA, BD Butler: Surfactants identified on the pericardium and their ability to impart boundary lubrication. *Ann. Biomed. Eng.* 13 (1986), 573-586.
10. Hills, BA, BD Butler: Surfactants identified in synovial fluid and their ability to



- act as boundary lubricants. *Ann. Rheum. Dis.* 48 (1984), 51-57.
11. Davson, H: *A Textbook of General Physiology*, 3rd Ed., Churchill, London (1964).
  12. Singer, SJ, GL Nicolson: The fluid mosaic model of the structure of cell membranes. *Science* 175 (1972), 720-731.
  13. Jacobsson, S, I Kjellmer: Flow pattern and protein content of lymph in resting and exercising skeletal muscle. *Acta Physiol. Scand.* 60 (1964), 278-285.
  14. Folch, J, I Ascoli, M Lees, et al: Preparation of lipid extracts from brain tissue. *J. Biol. Chem.* 191 (1951), 833-841.
  15. Folch, J, M Lees, GH Sloane-Stanley: A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226 (1957), 497-509.
  16. Rouser, G, AN Siakotos, S Fleischer: Quantitative analysis of thin-layer chromatography and phosphorus analysis of spots. *Lipids* 1 (1966), 85-86.
  17. British Standards Institute. *Methods of Test for Textiles. British Standards Handbook*, London: H.M. Stationary Office, Vol. II, (1974), 3/46.49.
  18. Hills, BA: Water repellency induced by pulmonary surfactants. *J. Physiol.* 325 (1982), 175-186.
  19. Blodgett, KB: Films built by depositing successive monomolecular layers on a solid surface. *J. Am. Chem. Soc.* 57 (1935), 1007-1022.
  20. Gaines, GL Jr.: Transfer of monolayers to solids. Multilayers. In: *Insoluble Monolayers at Liquid-Gas Interfaces*, Ch. 8, Wiley, New York (1966), 326-346.
  21. American Society for Testing Materials. Standard test method for yield strength of performed sealing tapes. Designation C908-79. ASTM, Philadelphia (1979).
  22. Spector, WS: *Handbook of Biological Data*, Saunders, Philadelphia (1956), 52-62.
  23. Sherman, IA: Interfacial tension effects in the microvasculature. *Microvasc. Res.* 22 (1981), 296-307.
  24. Adamson, AW: *Physical Chemistry of Surfaces*, 2nd Ed., Wiley, New York (1967).
  25. Pattle, RE, C Schock, JM Creasey: Mitochondrial lamellated bodies and surfactant depots in the type II cells of the lung compared by bimetallic staining and X-ray analysis. *Brit. J. Exper. Path.* 61 (1980), 160-163.
  26. Hills, BA, BD Butler, RE Drake: Surfactants identified in lung lymph and their ability to act as adhesives. *J. Appl. Physiol.* 58 (1985), 514-520.
  27. Peeters, H: *The Lipoprotein Molecule*, Plenum, New York (1978).
  28. Bishop, WR, Y Kawashima, RM Bell: Membrane phospholipid bilayer assembly: Phospholipid biosynthetic enzymes and phospholipid transporters. *J. Neural Transmission (suppl.)* 24 (1987), 229-237.
  29. Hardy, WB: *Collected Papers of Sir William Hardy*, Cambridge U.P., Cambridge (1936).
  30. Boyd, J, AA Raimondi: Hydrodynamic lubrication: Fundamental requirements. In: *Handbook of Lubrication Engineering*, O'Connor, JJ, J Boyd, EA Avallone (Eds.), McGraw-Hill, New York (1968), 3.1-3.16.
  31. Hern, JF: Lubricants. In: *Paper Coating Additives*, Landes, CG, L Kroll (Eds.), Atlanta: Technical Association Pulp and Paper Industry (1978), 80-105.
  32. Guyton, AC: *A Textbook of Medical Physiology*, 3rd Ed., Saunders, Philadelphia (1966), 550.
  33. Kragh, AM, J Wooton: Animal glue and related protein adhesives. In: *Adhesion and Adhesives*, Houwink, R, G Salomon (Eds.), Elsevier, Amsterdam (1965), 140-1660.
  34. Drake, RE, JH Smith, JC Gambell: Estimation of the filtration coefficient in intact dog lungs. *Am. J. Physiol.* 238 (Heart Circulation Physiology 7) (1980), H430-H438.
  35. Drake, RE, DK Adcock, RL Scott, et al: Effect of outflow pressure upon lymph flow from dog lungs. *Circ. Res.* 50 (1982), 865-869.

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