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PULMONARY SURFACTANT: HYDROPHOBIC NATURE OF THE MUCOSAL SURFACE OF THE HUMAN AMNION

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SUMMARY

1. The contact angle has been measured for a drop of saline placed upon the rinsed mucosal surface of the amnion in eleven human placental membranes obtained from normal births at full term.

2. The contact angle averaged 70°, indicating a hydrophobic surface comparable with graphite (86°), polyethylene (94°) or oxyntic tissue (85°) which is also exposed to endogenous surface-active phospholipids *in vivo*.

3. By comparison, four pre-term placentas with an average gestation period of 29·5 weeks gave a mean contact angle of 32°, indicating that hydrophobicity of the placenta increases with maturity (41 weeks) and might well be imparted by adsorbed surfactants present in amniotic fluid and known to render other surfaces hydrophobic.

4. Since the mucosal epithelium of the amnion is exposed to the same surfactants in the same physical state as the fetal alveolar wall, the above results imply that this surface may also be hydrophobic, as indicated in the adult lung by other studies.

5. The concept of surfactant directly adsorbed to the pulmonary tissue surfaces is discussed in connexion with its possible functional advantages in 'de-watering' the lung at birth, maintaining homeostasis by water repellency, releasing airway surfaces and lymph ducts glued by protein and lubricating tissue respiratory movement.

INTRODUCTION

Since von Neergaard (1929) found the recoil of the excised lung to be greatly reduced by liquid filling, each alveolus has been assumed to be lined with a continuous aqueous hypophase as though it were the inside of a bubble. Surfactant can then locate at the air–aqueous interface to reduce surface tension and, hence, the pressure resulting from the inherent tendency of all bubbles to collapse, a tendency with potentially undesirable consequences for both pulmonary mechanics and homeostasis which have been reviewed by Clements & Tierney (1965). Considerable theory has evolved in developing a purely detergent role for surfactant in avoiding these hypothetical problems introduced by the model itself, e.g. alveolar instability.

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An alternative approach has been to argue that, in the normal physiological state, it would be more desirable teleologically if there were no bubble to collapse in the first place and, moreover, no liquid lining to impede gas exchange (Hills, 1981, 1982). While there is little doubt that a continuous liquid lining occurs in the fetus at birth or in infants with respiratory distress syndrome (r.d.s.) (Scarpelli, Jumar & Clutario, 1983), some proponents of the bubble theory, notably Pattle (1966), have expressed their concern at the failure of almost all morphological studies to demonstrate this lining in the adult mammalian lung. Subsequent morphological studies using different fixation methods, e.g. vascular perfusion procedures, continue to show the aqueous hypophase not as a continuous lining but largely confined to the septal corners as what Weibel & Bachofen (1979) term 'pools'.

This concept raises the question of what factors can be keeping the primary gas transfer surfaces essentially dry and establishing these dry patches soon after birth or re-establishing them after alveolar flooding. One possibility is that surfactant can be directly adsorbed to the mucosal surface just as it can be adsorbed to non-biological surfaces (Gershfield, 1979; Hills, 1982) and to cutaneous and tracheal epithelium (Hills, 1982; Hills & Barrow, 1979) to render those surfaces hydrophobic and, hence, water repellent. Electron micrographs used to determine the location of surfactant in the alveolus by Gil & Weibel (1969) and Weibel & Gil (1968) show osmium densities as high at the mucosal surface as any seen at the air–aqueous interface of the 'pools'. These indications of adsorbed surfactant are likely to be conservative since, as Untersee, Gil & Weibel (1971) point out, any fixation process destroys hydrophobic layers.

Molecular models for the direct adsorption of the primary components of pulmonary surfactant to the alveolar mucosa have been discussed elsewhere (Hills, 1982, 1983*b*) while their ability to act as water repellents has been demonstrated *in vitro* in two modes. First, they can inhibit fluid in deeper structures from exuding onto that surface (Hills, 1982) and, secondly, they can cause fluid already in contact with the surface to move aside, i.e. 'de-watering', including the ability to cause spontaneous rupture of continuous layers of adjacent fluid to expose dry surface *de novo* (Hills, 1983*a*). Any ability of adsorbed surfactants to establish dry patches on the alveolar wall of the new-born would obviously be highly desirable. The vital question is whether they possess this capability when coating the pulmonary membrane and, in turn, whether they are adsorbed to it when present in the physical form in which those surfactants are actually produced in the fetal lung. The phospholipid extracts from amniotic fluid into which those secretions flow have been shown to impart those properties *in vitro* (Hills, 1983*a*) but do they do so *in vivo* and in the human in particular?

In utero, the tracheal effluent enters amniotic fluid to provide the same surfactants in the same physical form as the fluid bathing the fetal alveolar epithelium. It can then be argued that, if these surfactants are adsorbed to the mucosal surface of the amnion to render it hydrophobic, then it is most likely they would do the same at epithelial surfaces in the fetal lung. In fact, it could be more hydrophobic than the amnion since, as Biggs, Hemming & McGeary (1974) have shown, the phospholipid concentrations in tracheal aspirates are an average of 11 times higher than in amniotic fluid, while the composition at amniotomy remains essentially the same (Giudicelli,

Pecquery, Magyar, Lacasa & Nordmann, 1975). This study has therefore been designed to detect any hydrophobicity of the amniotic surface of placental membrane as in *in vivo* indication of the capability of fetal pulmonary surfactants to do the same at the fetal alveolar surface. This study has additional clinical relevance in that it can be undertaken using human material and has been extended to several placental membranes obtained from cases of premature delivery in an effort to identify any relationship between hydrophobicity and the respiratory distress syndrome.

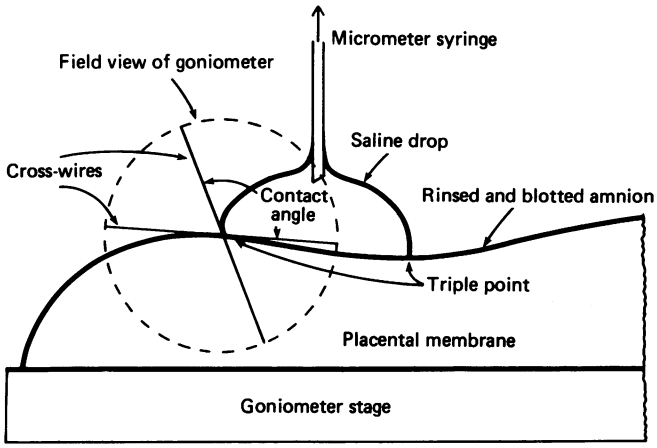

Fig. 1. Displaying the method of measuring contact angle by rotating cross-wires in the magnified field of view of a goniometer.

METHODS

Principle

It can be argued that any substances remaining on the mucosal surface of the amnion after rinsing well with saline must be either directly adsorbed or sufficiently strongly attached by other means for the nature of the bond not to be a major concern. It is particularly significant if the resulting surface is then found to be hydrophobic.

Hydrophobicity is most easily characterized by any tendency of an aqueous fluid to form beads on the surface rather than to spread evenly as it would on a perfectly wettable surface. The simplest quantitative index for this phenomenon is the contact angle (Adamson, 1967). This is the angle between the solid–liquid and liquid–air interfaces at the triple point where all three phases meet. It is strictly defined by the Young equation relating it to surface energy, but offers a simple index of the hydrophobic nature of a surface. It is zero for hydrophilic, wettable surfaces such as glass or most untreated tissues such as venous endothelium which needs to be dried for 20 min before displaying any contact angle (Sherman, 1981).

Materials

Human placentas (term and pre-term) were collected from the delivery room within 5 min of the third stage of delivery and immersed in saline, taking care to avoid contamination of the amnion by blood. These were taken to the laboratory where membrane sections 2 cm × 10 cm were cut and very well rinsed with a large excess of saline. Each membrane section was placed on the flat horizontal stage of a goniometer, which is the standard instrument for measuring contact angle, and the same procedure followed as described in detail by Hills, Butler & Lichtenberger (1983) for other tissue surfaces coated with endogenous surfactant.

Contact-angle measurements

After vigorous rinsing any excess saline was removed by gently blotting at 25 °C. The instrument used for measuring contact angle was a goniometer (Ramé-Hart model 100-00 115) widely employed by cosmetic chemists for making such measurements on human skin. The instrument was fitted with a monochromatic light source, camera attachment and micrometer-activated syringe (Ramé-Hart model 100-10) for applying small volumes of saline to the tissue surface. Five microlitres of normal saline were applied to the amniotic surface and the contact angle measured in the usual way.

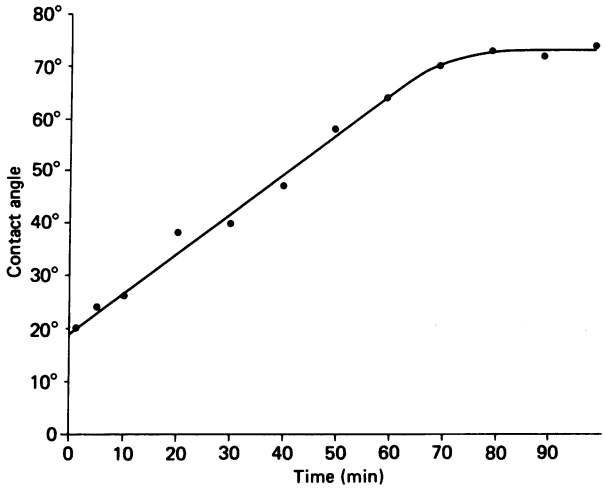

Fig. 2. A typical relationship between the contact angle subtended by a drop of saline placed on the mucosal surface of human amnion and the time elapsed since gently blotting to remove rinsing solution.

The centre of the field of view was adjusted to coincide with the triple point and then one cross-hair was adjusted to coincide with the tissue–fluid interface while the other was orientated until it represented a tangent to the liquid–air interface. The angle between the two cross-hairs now represents the contact angle (see Fig. 1) and can be read off from the scale encircling the eye-piece. Magnification ($\times 25$) of the triple point enables the observer to allow for tissue irregularity in measuring contact angle. The effects of micro-irregularities is a subject of discussion by physicists, e.g. Mason (1978), but the macro value is still regarded as a good reflexion of the micro value. Contact angle determinations were repeated at two sites on each of three samples taken from each placental membrane.

RESULTS

The epithelial surface of the amnion from each of eleven full-term births all showed an appreciable contact angle (θ) when measured within 1 min of gently blotting the surface. This angle increased when measured on the same surface at 5 min intervals. A typical curve is shown in Fig. 2 from which it can be seen that θ reaches a plateau after about 75 min. Plateau values for all ten placentas from normal births occurring at term are given in Table 1. The pre-term placentas included were all of those occurring in the delivery room during the period of the study.

TABLE 1. Contact angle of saline on the mucosal surface of the amnion

Placenta	Contact angle (θ) Initial value	Plateau value	Gestation period (weeks)
1	38°	76°	42
2	14°	54°	42
3	17°	44°	40
4	14°	90°	40
5	20°	70°	42
6	18°	48°	42
7	26°	80°	39½
8	20°	73°	41
9	20°	70°	41
10	26°	75°	40
11	27°	92°	41
Term mean	21·8°	70·2°	40·9
S.E. of mean	±2·2°	±5·0°	±0·3
12	19°	31°	26
13	14°	42°	29
14	16°	32°	31
15	15°	23°	32
Pre-term mean	16·0°	32·0°	29·5
S.E. of mean	±1·0°	±3·9°	±1·3

DISCUSSION

The results show that the mucosal lining of the human amnion at term displays an appreciable contact angle, averaging 22° immediately after rinsing and gently blotting. When adhering surface water is allowed to evaporate, this rises to plateau values averaging 70° (see Table 1) which can be compared with those for surfaces well known for their hydrophobic properties (Adamson, 1967) such as graphite (86°), polyethylene (94°) and Teflon (106°). A placental contact angle of 70° is large by comparison with tissues generally considered hydrophilic such as 20° for the serosal side of gastrointestinal tissue (Hills *et al.* 1983) or venous endothelium where the initial contact angle is 0° but rises to a plateau value of 23° according to Sherman (1981). That author considers even an angle of 23° to reflect hydrophobic characteristics. Hence there is little doubt that the mucosal surface of the amnion can be regarded as hydrophobic, especially at fetal maturity.

The contact angle of 70° is similar to values of up to 67° for exogenous dipalmitoyl lecithin on tracheal epithelium as deduced from surface tension studies using a Langmuir trough by Hills & Barrow (1979) or those measured directly by a goniometer *in vitro* (Hills, 1983*b*). It is, however, rather lower than a mean value of 84° recorded on oxyntic tissue by Hills *et al.* (1983), but within the general range of gastrointestinal tissues on which endogenous surfactant has been identified and characterized (Butler, Lichtenberger & Hills, 1983).

If surface-active phospholipids are adsorbed to the mucosal surface of the amnion

in a manner similar to that proposed in the studies quoted above, then they are most probably responsible for its hydrophobic nature. The tissue itself is unlikely to be hydrophobic since the pre-term placentas had much lower contact angles, indicating that the rise in θ is a function of fetal maturity probably mediated via increasing surfactant availability and adsorption. This conclusion is further supported by the fact that lipid solvents such as chloroform virtually eliminated the contact angle, as did bile salts which have the same effect on oxyntic tissue (Hills *et al.* 1983).

Since, *in utero*, the fetal alveolar mucosa and the mucosal surface of the amnion are exposed to the same surfactants in essentially the same physical state, it is quite likely that the two surfaces would acquire a similar coating. If there is any difference due to surfactant, the fetal alveolar mucosa is likely to be more hydrophobic since it is closer to the source of the more surface-active, saturated phospholipids and exposed to them at a much higher concentration, about 11-fold higher according to Biggs *et al.* (1974). Hence this study indicates but does not prove that the fetal alveolar mucosa is likely to be quite hydrophobic at fetal maturity yet appreciably more hydrophilic with decreasing gestational age. This could be related to the observation of Barrow & Hills (1983) that the contact angle induced by dipalmitoyl lecithin (DPL) and, hence, the hydrophobicity imparted by DPL can be potentiated by the addition of some dipalmitoyl phosphatidylglycerol (DPPG). This anionic surfactant appears in amniotic fluid at a gestational age of about 34 weeks and rises to about 9–10 % of total surfactant at fetal maturity (Hallman, Kulovich, Kirkpatrick, Sugarman & Gluck, 1976). There is also the likelihood that amniotic fluid ingested by the fetus will tend to coat the lumen of the stomach and help to provide gastric cytoprotection in the infant, just as proposed for the adult (Hills *et al.* 1983) in which similar surfactants have been clearly identified along the gastrointestinal tract by Butler *et al.* (1983). It is tempting to speculate that a deficiency in surfactant could cause inadequate adsorption to both the alveolar and gastric mucosae of the infant at birth and this may explain the strong clinical correlation found between respiratory distress syndrome and necrotizing enterocolitis in the new-born (Amoury, 1980).

An essentially hydrophobic alveolar wall would have considerable advantages in 'de-watering' the alveolar surface just as surfactants with similar functional moieties are widely employed to perform similar functions industrially (ABM, 1979; British Standards Institute, 1966). Along with other functional advantages previously described (Hills, 1982, 1983*a*), dry patches would reduce the blood–air barrier to gas transfer. Adsorption may also explain the delay in the elevation of arterial P_{O_2} observed in infants with respiratory distress syndrome when exogenous surfactant is instilled into the airways despite the immediate rise in compliance (Morley, Bangham, Miller & Davis, 1981). Instilled surfactant in its active 'dry' form would act immediately in reducing surface tension at the air–aqueous interface on which it first impinges but could take several hours to transcend the continuous aqueous hypophase of the neonate with respiratory distress syndrome and undergo the slow process of adsorption to the alveolar wall.

Another aspect of adsorbed surfactant is its ability to allow airways to open, either by modifying surface tension (Sanderson *et al.* 1976; Riefenrath, 1983) or by acting as a release agent in the adsorbed state. In the latter case, adsorbed DPL and natural phospholipids recovered from the lining of Eustachian tubes have been shown to

reduce by 90–95% the force needed to separate surfaces glued together with protein (Hills, 1983*c*). There is the possibility that adsorbed surfactant could further aid the neonate in establishing normal respiration by acting as a boundary lubricant for airway motion in much the same way as proposed for pleural movement (Hills *et al.* 1982).

In conclusion, it can be said that adsorption of surfactants to the mucosal surfaces of the fetal lung implied by this study may be necessary to aid in establishing normal respiration.

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