INTRODUCTION

IT IS NOW WIDELY ACCEPTED that the moist respiratory surface of the mammalian lung is lined by a material which is responsible for lowering the surface tension at the air-fluid interface. This material, which is generally referred to as "pulmonary surfactant", appears to account for much of the mechanical stability of the terminal air spaces during the movements of respiration (Clements et al, 1958; Pattle, 1958). Research into the biophysical and biochemical properties of pulmonary surfactant has been spurred on by the discovery that surface tension is abnormally high in the lungs of infants dying from respiratory distress syndrome (Avery and Mead, 1959).

The morphological demonstration of pulmonary surfactant in situ has proven difficult. The widely used practice of preserving lung by introducing a solution of fixative through the bronchial tree causes the surfactant lining layer to be displaced. Displacement of surfactant also occurs when attempts are made to excise small blocks of tissue from the surface of unfixed lungs. Vascular perfusion of fixative, a technique often used in the preparation of organs for electron microscopy, is seldom successful when applied to lung tissue: the pulmonary vascular bed can only be perfused with fixative solutions at pressures far above the physiological range and as a result gross pulmonary oedema invariably develops. Furthermore, the surfactant lining is rapidly dissolved by the organic solvents which are used in the preparation of tissues for electron microscopy. Indeed, the results of a recent study (Meban, 1973) have suggested that chemical extraction has probably been the major cause of failure in previous attempts to visualize surfactant.

In this paper an account is given of a study in which pulmonary surfactant was successfully demonstrated in situ by electron microscopy using a modified technique for tissue preparation. The lung of the Syrian hamster was used as an experimental model: in this species the micro-structure of the lung closely resembles that of Man (Ryan et al, 1969) and, in addition, tissue samples can be obtained from adult and foetal animals in a fresh state—a technical necessity for electron microscopy.

MATERIALS AND METHODS

Foetal (10th-16th day of gestation), neonatal (air-breathing for 5 minutes-12 hours), and young adult (9 months old) Syrian hamsters were used. The animals were killed by cervical dislocation, the lungs were dissected out and immersed for 15 minutes in an ice-cold solution of 3 per cent glutaraldehyde in 0.1M cacodylate buffer (pH 7.35). Small blocks of tissue were then cut from the subpleural region
of each lung and fixed for a further 2 hours in the same solution. The blocks were washed for 18 hours in buffer solution containing 0.25M sucrose, post-fixed in buffered 2% osmium tetroxide, and rapidly dehydrated in ethanol. The schedule for dehydration was as follows: (1) 60% ethanol at 4°C (5 minutes); (2) 80% ethanol at 4°C (5 minutes); (3) absolute ethanol at 4°C (30 minutes); (4) absolute ethanol at room temperature (30 minutes). Following dehydration the tissues were processed through 1,2-epoxypropane and embedded in Araldite or Epon-Araldite. Sections (50-70 nm thick) were cut on a Reichert ultra-microtome, stained with uranyl acetate and lead citrate, and examined in an AEI electron microscope.

RESULTS

Lungs of adult animals

In electron micrographs of adult hamster lung a layer of pulmonary surfactant is seen to line the entire internal surface of the alveoli and alveolar ducts. In some specimens an incomplete surfactant lining is also present in the smaller bronchioles. The surfactant lining consists of two distinct parts: (1) a thin electron-dense superficial film, and (2) a thicker basal layer.

The superficial film extends as a gently curved line around the interior of each alveolus (Figure 1). The film is of uniform thickness (7 nm) and shows a strong affinity for heavy metal stains. When cut in true cross-section it is sharply delineated, but it is often indistinct in tangential sections. In some regions the film

![Figure 1](image-url)

**FIG. 1.** Surfactant lining in lung of adult hamster. AL, alveolar lumen; SF, superficial surfactant film; BL, basal layer of surfactant; AE, alveolar epithelium. x 130,000.
is fragmented and appears as a series of electron-dense linear masses. High resolution micrographs have failed to reveal any regular substructure within the film.

The basal layer occupies the space between the superficial film and the underlying alveolar epithelium (Figure 1). In the depressions between adjacent pulmonary capillaries and at the angulated areas of the alveolar wall this layer is several micrometres thick. In contrast, it is very attenuated (as little as 50 nm thick) over regions of the alveolar wall where gaseous diffusion occurs. The basal layer has a floccular appearance and it contains membranous structures and small numbers of alveolar macrophages. The membranous structures are composed of lamellae which resemble the superficial film in thickness and staining properties. The lamellae are generally disposed in an orderly manner as square lattices or parallel arrays (Figure 2); less frequently, loose tangles or whorls are formed. Macrophages are normally present only in the thicker regions of the basal layer. In most cases they appear to be actively engaged in the removal of particulate matter from the superficial surfactant film.

Lungs of foetal animals

Osmiophilic surfactant membranes first appear in the lungs of foetal hamsters on the 13th day of gestation. The membranes are scattered throughout the fluid contained within the terminal respiratory spaces. They are most often arranged in irregular tangles, although in some areas square tubular arrays or concentric cylindrical sheets can be detected (Figure 3). The quantity of the membranous material shows a sharp increase during the last two days of gestation (15th and 16th days).

FIG. 2. Adult hamster lung. Membranous material in form of a square lattice in basal layer of surfactant. x 80,000.

FIG. 3. Surfactant membranes in lung of foetal hamster. CL, concentric lamellae; SL, square lattice. x 80,000.
After birth the fluid within the respiratory passages is gradually replaced by air. During this process some of the surfactant membranes unite to form a continuous film at the newly formed air-fluid interface. Membranous material not used in this way is stored in the residual alveolar fluid and thus forms the basal, or reserve, layer of the surfactant complex.

**Discussion**

In the present study the layer of pulmonary surfactant that lines the terminal air spaces of hamster lung has been demonstrated in situ by electron microscopy. The morphological form of the lining layer corresponds closely to the structural model of surfactant proposed by Pattle (1966) on the basis of physiological and biochemical data.

Pattle (1966, 1967) has suggested that the superficial film of the lining complex consists of a monomolecular sheet of phospholipid molecules situated at the air-fluid interface in such a manner that the hydrophobic parts of the molecules are orientated towards the air phase. The basal layer is considered to be a colloidal solution of protein and phospholipid which serves as a depot from which the superficial film can draw during alveolar expansion.

Morgan and Huber (1967) have shown that 60-65 per cent of all lipids are lost during the processing of tissues for electron microscopy by conventional techniques; most of the loss appears to occur during the dehydration of the tissues in organic solvents. Obviously extraction of this order of magnitude greatly hinders the visualization of thin membranes, such as those of pulmonary surfactant, which have a high lipid content. In the present study the loss of lipid by chemical extraction was minimized by processing the tissues for electron microscopy by a rapid dehydration technique.

Gil and Weibel (1970) have had some success in preserving the surfactant lining in the lungs of rats. They perfused the pulmonary vascular bed with an anticoagulant (heparin) and a vasodilator (procaine) and then with a solution of glutaraldehyde fixative. In this way they were able to stabilize the alveolar tissue components without causing massive pulmonary oedema. Their micrographs clearly show a duplex type of surfactant lining similar to the one seen in the present study. Chase (1959) has attempted to demonstrate pulmonary surfactant using a freeze-drying technique. Unfortunately tissues prepared in this way show little contrast in the electron microscope and hence the results of this study are difficult to interpret.

In the present study surfactant membranes were detected in the terminal respiratory spaces of foetal hamsters as early as the 13th day of gestation. The membranes took the form of loose tangles or, less commonly, they were arranged in square lattices or concentric cylindrical sheets. The first appearance of a continuous alveolar lining film was seen immediately after birth when air was first introduced into the lungs. The presence of large quantities of a surface tension lowering agent in the air spaces at this time is undoubtedly of great functional benefit: the surfactant would facilitate the removal of fluid from the lung and also greatly decrease the effort required to perform the first respiratory movements.
It now appears likely that the regulation of alveolar surface tension is not the sole function of pulmonary surfactant. It is well established that phospholipid films in vitro are capable of absorbing particles of insoluble material (Fergason and Brown, 1968) and Fronsolono et al, (1970) have recently suggested that the surfactant film may assist in the trapping of inhaled particulate matter. On the other hand, Meban (1972) has shown that the basal layer of the surfactant lining is rich in mucopolysaccharides which exist in a highly hydrated form. It is therefore probable that this component of the lining complex protects the delicate alveolar epithelium from dessication.

SUMMARY

The pulmonary surfactant that lines the alveoli of hamster lung has been demonstrated in situ by electron microscopy. In the lungs of adult animals the surfactant lining consists of two distinct parts: (1) a thin superficial film, and (2) a thicker basal layer. Surfactant membranes first appear in the lungs of foetal hamsters on the 13th day of gestation. They are arranged either in loose tangles or in square lattices and concentric cylindrical sheets.

I am grateful to Mr. G. R. Dickson and Mr. M. S. Henderson for technical assistance, and to Miss A. Richardson for secretarial help. This study was supported by a grant from the Northern Ireland Hospitals Authority.

REFERENCES

AVERY, M. E. and MEAD, J. (1959). A.M.A.J.Dis. Child. 97, 517.
CHASE, W. H. (1959). Expl.Cell Res. 18, 15.
CLEMENTS, J. A., BROWN, E. S. and JOHNSON, R. P. (1958). J.appl.Physiol. 12, 262.
FERGASON, J. L. and BROWN, G. H. (1968). J.Amer. Oil Chem.Soc. 45, 120.
FRONSOLONO, M. F., CHARMS, B. L., PAWLOSKI, R. and SLIVKA, S. (1970). J.Lipid Res. 11, 439.
GIL, J. and WEBEL, E. R. (1970). Resp.Physiol. 8, 13.
MEBAN, C. (1972). Histochem. J. 4, 1.
MEBAN, C. (1973). "Ultrastructure and Cytochemistry of Lung Alveoli". M.D. Thesis, Queen's University of Belfast.
MORGAN, T. E. and HUBER, G. L. (1967). J.Cell Biol. 32, 757.
PATTLE, R. E. (1958). Proc.Roy.Soc. London (Ser. B) 148, 217.
PATTLE, R. E. (1966). In "Advances in Respiratory Physiology" (Ed. C. G. Caro), Edward Arnold: London.
PATTLE, R. E. (1967). In "Development of the Lung" (Ed. A. V. S. de Reuck and R. Porter). p. 173. Churchill: London.
RYAN, S. F., CIANNELLA, A. and DUMAIS, C. (1969). Anat. Rec. 165, 467.