being used as dehydrator and thinner with a quick time-schedule (Schock *et al.* 1973). The bodies are characterized by a marked layering with 4 nm spacing These methods demonstrate no obvious difference between LOPBs from various mammals and from the lungfish *Lepidosiren*; we have found no sign of the marked species differences reported by Kikkawa & Spitzer (1969). LOPBs appear to be rare in, but not absent from, newt lung.

REFERENCES
Avery M E & Mead J (1959) *American Medical Association Journal of the Diseases of Children* **97**, 517
Dermer G B (1969) *Journal of Ultrastructure Research* **27**, 88
Hughes G M
(1966) *Proceedings of the Royal Society of Medicine* **59**, 494
(1967*a*) Comparative Physiology of Vertebrate Respiration. Heinemann, London
(1967*b*) In: Development of the Lung. Ed. A V S de Reuck & R Porter. Churchill, London; pp 64–80
Jarvik E (1966) *Arkiv för Zoologie* **19**, 41
Kikkawa Y & Spitzer R (1969) *Anatomical Record* **163**, 525
Klaus M, Reiss O K, Tooley W H, Piel C & Clements J A (1962) *Science* **137**, 750
Parsons T S & Williams E E
(1963) *Quarterly Review of Biology* **38**, 26
Pattle R E
(1965) *Physiological Reviews* **45**, 48
(1969) In: Fœtal Autonomy. Ed. G E W Wolstenholme and M O'Connor. Churchill, London; p 132
Pattle R E & Hopkinson D A W (1963) *Nature (London)* **200**, 894
Pattle R E, Schock C & Creasey J M (1972) *Experientia* **28**, 286
Rossdale P D, Pattle R E & Mahaffey L W (1967) *Nature (London)* **215**, 1498
Schock C, Pattle R E & Creasey J M (1973) *Journal of Microscopy* **97** (in press)
Thomson K S (1967) *Journal of Paleontology* **41**, 660

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Electron Microscopic Aspects of Surfactant Secretion

Two cell types have been suggested as the source of pulmonary surfactant:

(1) *The Type II pneumonocyte* or great alveolar cell – a cuboidal cell that contributes to the alveolar lining: it has cytoplasmic projections at its free apical edge and contains characteristic osmiophilic, lamellated bodies (Fig 1).
(2) *The nonciliated cell* or 'Clara' cell of the airways containing smooth endoplasmic reticulum in its cytoplasm and dense, homogeneous, non-osmiophilic granules at the apical edge.

Macklin (1954) was responsible for the first suggestion and Niden (1967) for the second.

The following evidence, though mainly circumstantial, makes a stronger case in favour of the Type II pneumonocyte than of the non-ciliated cell:

(*a*) In the fœtal lung the lamellated bodies appear first at the same time as surfactant activity. On electron microscopy the Type II pneumonocytes are first recognized in the fœtal rabbit lung, on the 27th day, by their cuboidal shape and lamellated bodies (Reid & Meyrick 1969). With physiological methods an increase in lung distensibility is found between the 27th and 28th days of fœtal life, due to the appearance of surfactant (Humphreys & Strang 1967).
(*b*) Klaus *et al.* (1962) found that bilateral cervical vagotomy of guinea-pigs produced a rise in pulmonary surface tension accompanied by a reduction in number of lamellated bodies.
(*c*) Schaefer *et al.* (1964) exposed guinea-pigs to 15% CO_2 in air and found abnormal vacuolated bodies and a rise in pulmonary surface tension: both revert to normal when the animal is returned to a normal atmosphere.
(*d*) An appearance suggesting discharge of the lamellated bodies from the Type II pneumonocyte into alveoli is seen. This could also be interpreted as absorption, but the complicated structure of the lamellated bodies makes this unlikely. The nonciliated cell is not seen to discharge granules in this manner.
(*e*) Chloroform/metranol extraction removes the lamellated bodies from the Type II pneumonocyte and the surfactant layer from the alveolus (Kikkawa *et al.* 1970). Askin & Kuhn (1971) did a similar experiment after tritiated palmitate, a

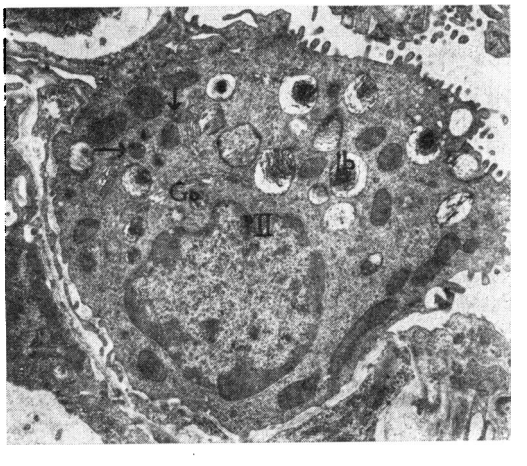

Fig 1 *A Type II pneumonocyte* (II) *showing Golgi apparatus* (Go), *multivesicular bodies* (↑) *and characteristic lamellated bodies* (lb). *Glutaraldehyde + osmium tetroxide: lead hydroxide.* × *5300*

labelled fatty acid, had been injected intraperitoneally into rats. They show, using scintillation counts and thin-layer chromatography, that 95% of the lipid lost is phospholipid, the type found in surfactant. The nonciliated cell granules remain unchanged, except for the loss of their limiting membranes.

(*f*) Wang *et al.* (1971) gave 9-fluoroprednisolone to fœtal rabbits at 24 days gestation. This accelerated the maturation of the Type II pneumonocyte, which at 26 days appeared morphologically as mature as the control rabbits of 28 and 29 days. The maturation of the nonciliated cell of the airway was unaltered. Physiological methods showed a decrease in surface activity by the 27th day (Kotas & Avery 1971).

(*g*) Askin & Kuhn (1971) also traced lipid synthesis, using tritiated palmitate with electron microscopic autoradiography. The majority of grains lay over the Type II pneumonocyte, with a lesser concentration over the nonciliated cell, indicating a faster lipid turnover in the Type II pneumonocyte than in the nonciliated cell.

The above is the evidence linking the Type II pneumonocyte with the synthesis and secretion of surfactant.

Niden's (1967) evidence in support of the nonciliated cell of the airway being responsible for surfactant production is the presence of phospholipids within the cells and the rapid uptake into the apical edge of the cell of tritiated palmitate and acetate, with a lesser amount into the Type II pneumonocyte; however, this must be reassessed in the light of Askin & Kuhn's study cited above. Niden also challenged the alveolar epithelium with carbon particles and found that the particles were ingested by the Type II pneumonocyte: he therefore suggested that this cell was the site of surfactant degradation. After intratracheal administration of Thorotrast, Corrin (1970) was also able to demonstrate a limited phagocytic activity in the Type II pneumonocyte as well as in the Type I pneumonocyte, but the macrophages ingested much more than either.

Since the function of surfactant is mainly alveolar the Type II pneumonocyte has the further advantage of being situated within the alveolus, unlike the nonciliated cell of the airway. Although surfactant may help to maintain the patency of the small airways it is difficult to imagine that fluid secreted in the airways would flow back into the alveolus against the ciliary beat. In man, fluid formed in the airways might have to travel distances up to 1 cm to reach the edge of the acinus.

The origin of the lamellated bodies is not yet established but several mechanisms have been suggested:

(1) Kisch (1955) first postulated that lamellated bodies are transformed mitochondria. More recently Pattle (personal communication) has shown that certain stimuli cause the appearance of transition bodies intermediate between mitochondria and lamellated bodies. These do occur in the normal animal, but are rare.

(2) Sorokin (1966) considered that lamellated bodies originate from multivesicular bodies, an organelle found in many cells and originating in the Golgi apparatus. He supported his suggestion with a series of convincing micrographs.

(3) Kikkawa *et al.* (1968) used fœtal rabbit lungs for their study and found transition forms, again beginning in the Golgi apparatus, between dense bodies and lamellated bodies. More recently Kikkawa & Spitzer (1969) have shown regions where multivesicular and dense bodies appear to fuse, thus bringing together the last two theories.

(4) Large pools of glycogen are seen in the fœtal lungs of the rat (O'Hare & Sheridan 1970), rabbit and man. Membranes from within these possibly represent precursors of the lamellated body.

The lamellated body may arise in a variety of ways, but in our studies the transition forms between the multivesicular and lamellated body are most frequently encountered.

Niden (1967) considers that the nonciliated cell granules also originate in the Golgi apparatus. In our department we have not found that this is obvious in the normal animal.

As evidence stands at present, it is thought that the Type II pneumonocyte plays the main part in surfactant production and secretion, but it may be that the nonciliated cell of the airways has a secondary or supporting role in the surfactant story.

REFERENCES

Askin F B & Kuhn C (1971) *Laboratory Investigation* **25**, 260
Corrin B (1970) *Thorax* **25**, 110
Humphreys P W & Strang L B (1967) *Journal of Physiology* **192**, 53
Kikkawa Y, Hahn H, Yang S & Bernstein J (1970) *Laboratory Investigation* **22**, 272
Kikkawa Y, Motoyama E K & Gluck L (1968) *American Journal of Pathology* **52**, 177
Kikkawa Y & Spitzer R (1969) *Anatomical Record* **163**, 525
Kisch B (1955) *Experimental Medicine and Surgery* **13**, 101
Klaus M, Reiss O K, Tooley W H, Pitt C & Clements J A (1962) *Science* **137**, 750
Kotas R V & Avery M E (1971) *Journal of Applied Physiology* **30**, 358
Macklin C C (1954) *Lancet* i, 1099
Niden A H (1967) *Science* **158**, 1323
O'Hare K H & Sheridan M N (1970) *American Journal of Anatomy* **127**, 181
Reid L & Meyrick B (1969) *Poumon et le coeur* **25**, 201
Schaefer K E, Avery M E & Bensch K (1964) *Journal of Clinical Investigation* **43**, 2080
Sorokin S (1966) *Journal of Histochemistry and Cytochemistry* **14**, 884
Wang N S, Kotas R V, Avery M E & Thurlbeck W M (1971) *Journal of Applied Physiology* **30**, 362