DEVELOPMENT OF INTERCELLULAR JUNCTIONS IN THE PULMONARY EPITHELIUM OF THE FOETAL LAMB

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SUMMARY

The integrity of epithelial tight junctions in foetal mammalian lungs is essential to maintain the unique ionic composition of lung liquid, and to prevent leakage of serum proteins into peripheral air spaces. In the present study the development of intercellular junctions of the lining epithelium of foetal lamb lungs during gestation was examined by light and electron microscopy. Both thin sections and freeze-fracture replicas were examined by electron microscopy. By 39 days of gestation, epithelial tight junctions consist of a minimum of $3.1 \pm 1.6$ (S.D.) and a maximum of $5.8 \pm 2.0$ discontinuous rows of particles and short segments of strands on P face ridges and in complementary E face grooves, while from 58 to 76 days they are composed of a network of $4.3 \pm 1.6$ to $7.7 \pm 1.9$ focally interrupted P face strands. Complementary replicas show that many of the discontinuities on the P face are due to separation of junctional particles on to the E face during fracturing, and not to an absence of junctional particles. From 76 days to term, epithelial tight junctions (exclusive of upper airway epithelium which was not examined) resemble those of adult lungs, and consist of a continuous network of $4.5 \pm 2.0$ to $7.5 \pm 2.5$ P face strands and complementary particle-free grooves. Permeability measurements, published elsewhere, indicate that the epithelium is functionally 'tight' from 69 days onwards. Tight junctions in peripheral air-space epithelium, therefore, are structurally continuous and functionally 'tight' early in foetal lung development, and form seals at one end of long, narrow intercellular spaces; these features may be important for coupled ion and water transport. When the bounding epithelial cells become flattened, these narrow intercellular spaces remain intact as a result of complex interdigitations of adjacent cell membranes. Desmosomes were present throughout gestation near the abluminal side of the tight junctions and occasionally near the base of the intercellular space. These junctions may serve to connect cells to each other at a time when tight junctions may be mechanically weak. In addition, gap junctions are associated with tight junctions from the glandular through the canalicular stages of lung development. They disappear by 120 days when the epithelial cells are differentiated.

INTRODUCTION

Lungs of foetal mammals are filled with a liquid of unique ionic composition and low protein content (Adams, Moss & Fagan, 1963; Adamson, Boyd, Platt & Strang, 1969) which is neither plasma ultrafiltrate nor amniotic fluid, nor an admixture of the two. That this fluid is elaborated in the lung itself was suggested by the experiments of Jost & Policard (1948), in which tracheal ligation in foetal rabbits resulted in the progressive distension of the lungs by liquid. While the functions of this complex liquid in foetal lungs are at present unclear, it seems reasonable to suggest that, at least in part, it serves to prevent developing air spaces from collapsing, and that the
secretory cells lining the lung play a role in controlling the degree of distension and growth (Alcorn et al. 1977).

The unusual ionic composition of this fluid, with its high Cl⁻ and K⁺ and low HCO₃⁻ concentrations, has been shown to be the result of active transport of these ions between interstitial fluid and lung liquid. Of these the most important, in terms of lung liquid secretion, is Cl⁻. Bulk active transport of this ion from interstitium to lung lumen, together with the passive movement of Na⁺ as counterion, provides the osmotic driving force required for water movement (Olver & Strang, 1974). In order to maintain both the disequilibrium in ion concentrations between interstitium and alveolar lumen and to allow efficient liquid secretion, it is necessary that a barrier, relatively impermeable to solutes, be maintained between these two compartments. Both physiological (Diamond, Barry & Wright, 1971; Frizzell & Schultz, 1972; Frömter, 1971; Frömter & Diamond, 1972) and ultrastructural (DiBona, 1972; DiBona & Civan, 1973; Machen, Erlij & Wooding, 1972; Whittembury & Rawlins, 1971) evidence suggests that tight junctions between epithelial cells are sites of low electrical resistance which provide a pathway for the passage of water, ions and small non-electrolytes, but effectively prevent the leakage of water-soluble macromolecules (Miller, 1960; Farquhar & Palade, 1963; Goodenough & Revel, 1970; Schneeberger & Karnovsky, 1968, 1971). If the same is true of the foetal alveolar epithelium, estimates of pore radius, based as they are on permeability measurements, should reflect the 'tightness' of the intercellular junctions. In the case of the mature foetal lamb, permeability measurements made late in gestation (123-143 days) indicate that the alveolar epithelium has the properties of an idealized membrane penetrated by water-filled cylindrical pores of 0.5-0.6 nm radius (Normand et al. 1971).

Most of the studies on lung liquid have been conducted in foetal lambs late in gestation when the lining epithelium is advanced in differentiation (Adamson et al. 1969; Boston et al. 1968; Normand et al. 1971; Olver, Reynolds & Strang, 1973; Olver & Strang, 1974). At present there is little information concerning either the permeability or the ultrastructure of the lining epithelium and its intercellular junctions at early stages of lamb lung development. The latter is of considerable interest both with regard to the morphogenesis of tight junctions, and the question of how their development regulates the permeability of the pulmonary epithelium, and hence the composition of lung liquid.

In the present study the ultrastructure of the peripheral air space epithelium and its intercellular junctions was examined in lungs obtained from foetal lambs between 39 days' gestation and term (147 days). From 69 days onward it was possible to obtain functional permeability measurements prior to fixation, which were correlated with the morphological observations on the same tissue after fixation (Olver, Schneeberger & Walters, in preparation). Although reliable permeability data were not available for foetal lungs taken prior to 69 days of gestation, junctions from very young foetal lambs (39 days onward) were examined by freeze fracture. Distinct differences in junctional morphology were evident in the young as compared to the older foetuses beyond 72 days of gestation.
MATERIALS AND METHODS

Lungs were obtained from 29 foetal lambs exteriorized at Caesarean section under chloralose anaesthesia, with the placental circulation intact. In a group of immature foetuses (69—76 days' gestation) permeability measurements were made prior to fixation of the lungs. Two inert radiolabelled tracers of differing size were introduced into lung liquid (see Olver et al. in preparation). For the most part the water-soluble non-electrolytes studied were [H]mannitol (a₀ = 0.42 nm) with [14C]urea (a₀ = 0.22 nm) (a₀ = corrected Stokes—Einstein molecular radius). Two other combinations were used: [14C]erythritol (a₀ = 0.35 nm) with [14C]fructose (a₀ = 0.31 nm) and [14C]erythritol with [14C]urea. In the latter case the two similarly labelled tracers were separated by Sephadex gel filtration. From the relative rates of disappearance of the two tracers in lung liquid, an estimate was made of the size of channels through which they passed while traversing the alveolar epithelium. The results were expressed in terms of an ideal membrane penetrated by cylindrical pores of uniform dimensions. (For full details of this technique and the method of data analysis, see Normand et al. 1971.) Immediately following completion of these measurements (or, when none was made, immediately following exteriorization of the foetus), lung liquid was withdrawn as completely as possible, and a similar volume of fixative composed of 13% formaldehyde and 1.6% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3 (Karnovsky, 1965), was instilled into the trachea. Care was taken not to over-distend the lung. Small pieces of lung (0.5 x 0.5 x 0.1 cm) were removed from the periphery, and in some instances from the hilar region, of the upper and lower lobes of the right and left lung respectively, and immersed in formaldehyde-glutaraldehyde fixative of the same concentration as had been infused. After fixation at 4°C for a period of 0.5—2 h the tissue was washed in 0.15 M cacodylate buffer, pH 7.3, and stored at 4°C.

For light and electron microscopy, small pieces (1 mm³) of tissue from both upper and lower lobes were postfixed in 1% OsO₄ with 15 mg/ml of potassium ferrocyanide (Karnovsky, 1971), for 1 h at 4°C. The tissue was stained en bloc with 1.5% uranyl acetate in 0.05 M maleate buffer, pH 6.2, dehydrated in graded ethanols, infiltrated and embedded in Epon. To correlate the freeze-fracture morphology of epithelial intercellular junctions with the various stages of lung development, 1-μm-thick sections from the upper and lower lobes of each lung were stained with toluidine blue and examined by light microscopy. For electron microscopy, thin sections were cut and picked up on carbon-coated grids, stained with lead citrate (Venable & Coggeshall, 1969) and examined in a Philips EM300 electron microscope. In order to determine the ultrastructural organization of epithelial junctional complexes, the surface configuration of the cell membranes, the degree of cellular differentiation, and to judge the quality of fixation, tissue from the upper and lower lobes of all animals was examined by transmission electron microscopy. In order to assess the length of intercellular spaces at various stages of development, a piece of string was aligned with the profile of one of the two cell membranes facing the intercellular space. The length of the string was then measured and recorded. For freeze fracture, 1-mm³ fragments of lung from upper and lower lobes, were infiltrated with glycerol at concentrations increasing from 10 to 30% in 0.1 M cacodylate buffer pH 7.3 for 2 h at 4°C, and rapidly frozen in liquid nitrogen cooled to −210°C under vacuum. The tissue was then fractured in a Balzers double replica device at −150°C in a Balzers high-vacuum freeze-etch unit. The carbon—platinum replicas were washed in Chlorox followed by distilled water, picked up on Formvar-coated grids and examined in the electron microscope. To ascertain, in the freeze-fracture replicas, that the junction examined was present in a peripheral air space, a low-magnification photograph of the latter was taken prior to the detailed examination of the contained junction(s). In selected lungs (before 74 days of gestation), care was taken to obtain, for examination, both halves of the fractured junction. That half of the junction facing the cytoplasm of the cell will be referred to as the protoplasmic or P face, while that half of the junction facing the extracellular space will be referred to as the exoplasmic or E face (Branton et al. 1975).

The present observations are limited to the peripheral air spaces, exclusive of upper airways.
RESULTS

Light microscopy

From 39 to 74 days of gestation the lungs consist of a small number of branching, tubular structures lined by a single layer of tall columnar epithelium, containing a large amount of glycogen primarily at the apices and bases of the cells (Fig. 1A). These tubular structures are surrounded by abundant loose mesenchyme containing a few, small, scattered, thin-walled blood vessels. From 74 to 94 days the branching tubular structures increase in number and become lined by a single layer of cuboidal epithelium filled with glycogen particles. Accompanying these changes there is a relative decrease in the amount of intervening mesenchyme. During this glandular stage of lung development no morphological difference can be detected in the degree of maturation between upper and lower lobes.

At 95 days, when the canalicular stage of development begins, an obvious difference in upper and lower lobe morphology becomes evident. In the lower lobes, closely spaced tubular structures lined by cuboidal epithelium persist (Fig. 1B), while in the upper lobes the air spaces become irregular and enlarged and are lined by a mixture of cuboidal and flattened epithelial cells, both of which contain a moderate amount of glycogen (Fig. 1C). This difference in the degree of upper and lower lobe differentiation persists until about 109 days of gestation, at which time both upper and lower lobes contain irregular air spaces lined by flattened cuboidal epithelium, consistent with the canalicular stage of development.

By 121 days of gestation, when the alveolar stage begins, developing alveolar structures appear in the upper lobes, whereas in the lower lobes irregular canalicular spaces persist. It is not until 130 days' gestation that both upper and lower lobes contain developing alveolar spaces (Fig. 1D) which are similar in appearance.

Transmission electron microscopy

Thin sections. From the 39th through the 69th day of gestation in the lamb, the cells lining the branching tubular structures in both upper and lower lobes are tall, columnar epithelial cells measuring up to 27 μm in height. They are bounded on the abluminal side by relatively straight unit membranes which show no basal infoldings, and have, on the luminal side, a few small irregular microvilli and a rare primary cilium (Fig. 2A). The cells contain a moderate amount of glycogen, nuclei with loosely organized chromatin, small numbers of randomly distributed mitochondria and little rough endoplasmic reticulum. The Golgi apparatus is small and occasional multivesicular bodies are observed. The adjacent cells are closely apposed, thus forming
long, narrow, straight intercellular spaces measuring 15–20 nm in width and averaging $22.2 \pm 5.2 \mu m$ in length (Table 1). Near the apex of the cells the extracellular space is obliterated by focal fusions of the adjacent cell membranes. These fusions are typical of tight junctions or zonulae occludentes. Towards the abluminal side of many of the tight junctions, one or rarely two desmosomes are seen (Fig. 2c). At the site of the desmosome, densely packed fibrils are attached to the cytoplasmic side of the cell membrane, and electron-dense, filamentous material fills the extracellular space.

Table 1. Length of intercellular space and height of adjacent cell in the lung during development of the foetal lamb

<table>
<thead>
<tr>
<th>Gestation, days</th>
<th>No. of intercellular spaces and cells measured</th>
<th>Height of adjacent cell, $\mu m \pm S.D.$</th>
<th>Length of intercellular space, $\mu m \pm S.D.$</th>
</tr>
</thead>
<tbody>
<tr>
<td>39–69</td>
<td>10</td>
<td>$20.4 \pm 7.2$</td>
<td>$22.2 \pm 5.2$</td>
</tr>
<tr>
<td>70–77</td>
<td>24</td>
<td>$13.7 \pm 1.8$</td>
<td>$19.8 \pm 3.4$</td>
</tr>
<tr>
<td>80–85</td>
<td>6</td>
<td>$9.6 \pm 1.3$</td>
<td>$16.3 \pm 2.8$</td>
</tr>
<tr>
<td>93–97</td>
<td>17</td>
<td>$4.5 \pm 2.2$</td>
<td>$9.7 \pm 3.6$</td>
</tr>
<tr>
<td>109</td>
<td>7</td>
<td>$0.6 \pm 0.5^*$</td>
<td>$1.4 \pm 1.3^*$</td>
</tr>
<tr>
<td>121–130</td>
<td>14</td>
<td>$0.3 \pm 0.1^*$</td>
<td>$0.9 \pm 0.6^*$</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>$0.6 \pm 1.8^*$</td>
<td>$3.3 \pm 1.6^*$</td>
</tr>
</tbody>
</table>

* Type I pneumocyte and its adjacent intercellular space.
† Type II pneumocyte and its adjacent intercellular space.

From 70 to 93 days of gestation, the lining columnar epithelial cells are not as tall as at earlier stages of gestation (Table 1). They contain large amounts of glycogen, small numbers of organelles, and a Golgi apparatus which is quite prominent in some cells. In contrast to earlier stages of development, there are several areas along some of the lateral cell surfaces, usually near the apex or base, in which adjacent cell membranes form complex interdigitations. Measurements of the length of intercellular spaces indicate that while they decrease in length, as gestation progresses, this decrease is proportionally less than the decrease in height of the adjacent cell (Table 1). The junctional complexes, consisting of tight junctions and desmosomes, are similar to those observed at earlier stages; however, some desmosomes are now also present.
towards the basal portion of the intercellular spaces. As at earlier stages of gestation, 
the cells lack basal infoldings, and the luminal membrane shows rare, irregular, short 
microvilli.

From 95 days onward, when irregular canalicular spaces form in the upper lobes, 
the lining epithelium is gradually reduced in height. The air spaces become lined by 
two types of epithelial cells or pneumocytes. Type I pneumocytes are present as 
flattened cells, but type II pneumocytes cannot be clearly identified until about 109 
days of gestation, when some of them contain multilamellar bodies within their 
cytoplasm. Small amounts of glycogen continue to be present within epithelial cells 
up to 130 days. As the lining cells become flattened, the adjacent lateral cell membranes 
form extensive, complex interdigitations which are primarily located near the base of 
the cells (Fig. 2B). From 109 days onwards, when type I and type II pneumocytes 
become differentiated, the length of the intercellular spaces is shortened. This shorten-
ing is more pronounced for the intercellular space between type I pneumocytes than 
between type I and type II pneumocytes, where it remains quite tortuous (Table 1). 
Desmosomes, which are mostly present on the immediate abluminal side of the tight 
junction, continue to be present towards the base of the lateral membrane as well.

Table 2. Number of junctional strands in epithelial tight junctions in the 
lung during development of the foetal lamb

<table>
<thead>
<tr>
<th></th>
<th>No. of junctions examined</th>
<th>No. of junctional strands</th>
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<tbody>
<tr>
<td>Group I</td>
<td>39-45</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.1 (±1.6)-5.8 (±2.0)</td>
</tr>
<tr>
<td>Group II</td>
<td>58-72</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.3 (±1.6)-7.7 (±1.9)</td>
</tr>
<tr>
<td>Group III</td>
<td>73-121</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5 (±2.0)-7.5 (±2.5)</td>
</tr>
</tbody>
</table>

* Minimum and maximum strand numbers in Group I are statistically different from the 
corresponding values in Groups II and III (P < 0.005). Strand numbers in Groups II and III 
are not statistically different.

By 130 days of gestation, the arrangement of the lateral cell membranes between 
type I pneumocytes begins to resemble that of the full-term lung; however, a single 
desmosome is often associated with tight junctions between both type I pneumocytes 
and between type I and type II pneumocytes. There is continued shortening of the 
length of the intercellular spaces, however, as at 109 days, the space between type I 
and type II pneumocytes remains somewhat tortuous and longer than between type I 
pneumocytes (Table 1). It is not until term is reached that desmosomes no longer 
form a part of the junctional complex.

Freeze-fracture replicas. At 39 days of gestation, the earliest stage examined, the P 
face of the tight junction between epithelial cells, of either upper or lower lobes, con-
ists of 3.1 ± 1.6 to 5.8 ± 2.0 discontinuous rows of particles or short segments of 
strands situated on low-profile ridges (Fig. 3A, C; Table 2). These junctional elements 
form a loose network, though some segments are completely separate from the main 
network. The luminal first and second rows of particles are continuous and it is
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Fig. 3. A. P fracture face of a tight junction from the RUL at 39 days of gestation. The junction is composed of interrupted rows of particles present on low ridges. Some of the rows of particles (arrow) are not connected to the main junctional network. The lumen is on the right.

B. E fracture face of a tight junction from the same RUL as in Fig. 3A. Numerous junctional particles are present in junctional grooves. A rounded cluster of irregular particles constitutes a desmosome (arrow). The lumen is on the right.

C. A segment of a tight junction, from the same lung as above, showing areas in which the junction is only one strand in width (small arrow). A small desmosome (d) is present in this area. An irregular segment of junctional strand is quite separate from the main junctional network (large arrow). The lumen is at the top. Arrowhead in circle, right lower corner, in these and all subsequent freeze-fracture micrographs indicates the direction of shadowing. All figs. x 82,500.
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primarily in the abluminal half of the junction that the disconnected segments of junctional elements are observed (Fig. 3A, c). Numerous junctional particles are present in the complementary shallow grooves on the E face (Fig. 3b). Intercellular junctions in foetal lamb lungs at 45 days' gestation are similar in appearance to those at 39 days' gestation. Complementary replicas made of these junctions show that the particles in E face grooves correspond to some of the small gaps between particles and short segments of strands on the P face ridges. This indicates that at least the 2 rows of particles near the lumen are continuous. However, on both P and E faces there are short segments of particle-studded ridges or grooves on the abluminal side of the junction respectively, which are not connected to the main network of the tight junction. This results in portions of the tight junction being only one or two rows of particles in width.

Fig. 5. Epithelial tight junction at 97 days gestation. Except for the presence of a few small clusters of particles resembling gap junctions (arrows), the continuous network of smooth strands is similar to that observed in adult lungs. x 54,000.

As gestation progresses (through 76 days), the rows of particles on the P face are replaced by a network of smooth strands, and fewer junctional particles remain in E face grooves. The number of strands comprising the junction from 58 days onwards is similar to that observed at the end of gestation (Table 2). A few defects within P face strands remain. However, using the double-replica technique it can be shown that from 69 days onwards, many of these discontinuities are due not to intrinsic defects of the junctional network, but rather to a tearing away on to the E face of portions of the junctional elements (Fig. 4A, B). It appears, therefore, that by 69 days of gestation many of the tight junctions are structurally continuous and, from the

Fig. 4. A. P fracture face of a tight junction in the RUL at 71 days of gestation. Small defects in the junctional strands are indicated by arrowheads.

b. Mirror image, E fracture face of the same tight junction as in a. Notice that in the complementary grooves, junctional particles (arrowheads) are present in the same areas as the defects in the strands on the P face. Both x 155,000.
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permeability measurements indicated below, they form functionally ‘tight’ permeability barriers.

From 76 days gestation onwards the pulmonary epithelial tight junctions of both upper and lower lobes fracture, as they do in full-term and adult mammalian lungs (Schneeberger & Karnovsky, 1976), i.e. a continuous smooth network of junctional strands is present on the P face (Fig. 3) and complementary, particle-free grooves are present on the E face. The number of junctional strands ranges from a minimum of $4.5 \pm 2.0$ to a maximum of $7.5 \pm 2.5$ at 73 days of gestation and remains unchanged throughout the remainder of gestation (Table 2).

Since, during lung development, the tubular structures lined by epithelium grow in a centrifugal pattern, and the upper lobes are at a more advanced stage of differentiation than the lower lobes, the following 2 questions were asked: (1) Is there any difference in the freeze-fracture morphology of tight junctions between upper and lower lobes? (2) Are there any differences in the morphology of tight junctions in tissue obtained close to the hilum as contrasted to that obtained near the pleura? As can be seen in Fig. 6A–D, there is little correlation between the number of strands constituting the junction, the way the junctional elements separate on to both P and E faces, and the location of the junction within the lung. Thus junctions at various stages of development and composed of varying numbers of strands are observed in each of the 4 locations from which tissue was obtained.

The earliest stage of gestation at which gap functions were observed was 58 days, when they are present within the meshwork of the tight junction strands. Occasionally they are seen immediately adjacent to the tight junction, abutting the most abluminal strand of the latter. More rarely a small cluster of gap junction particles is present at some distance from the tight junction (Fig. 6E). The gap junctions form tightly packed, hexagonal arrays of particles on the P face and complementary pits on the E face (Fig. 6C, E). While they are particularly numerous from 72 to 93 days of gestation, they are not present within all tight junctions, and from 93 days onwards they are seen less frequently and are smaller. They are absent from upper lobe junctions by 120 days as summarized in Fig. 7. Their mode of disappearance was not studied.

Fig. 6. A. Tight junction from the hilar portions of the RUL at 72 days of gestation. The junction is composed of 7–8 smooth strands.

B. Tight junction from the peripheral portions of the same lobe as in A. The junction is somewhat more particulate and contains few strands in cell (i) than in the adjacent cell (2).

C. Tight junction from the hilar portion of the RUL of the same lung as above. In some areas the junction is composed of only 2 strands and several gap junctions (arrows) are present.

D. Tight junctions from the peripheral portions of the RLL. The junctional network is continuous and composed of up to nine strands.

E. Tight junction in the LLL at 83 days of gestation. Numerous hexagonal arrays of gap junctional particles and complementary pits are present within the tight junctional network (small arrows). A single gap junction is also present by itself (large arrow). Notice that the E-face grooves of the tight junction are free of junctional particles. A, B, D, $\times 46000$; C, $\times 76000$; E, $\times 72500$.
Desmosomes are present most frequently on the abluminal side of the tight junctions. On both P and E faces they form rounded patches of particles which are irregular in size (Fig. 3B, c). While they are present on both P and E faces, the particles tend to be somewhat more numerous on the P face. As summarized in Fig. 7, desmosomes are present throughout gestation.

Permeability measurements

Epithelial permeability measurements were made in eight foetuses of 69–76 days' gestational age and the radius of equivalent pores calculated. Values ranged between 0.52 and 0.74 nm with a mean of 0.66 nm, which is similar to the figure of 0.64 nm for mature lambs (123–143 days' gestation) calculated by the same method from the data of Normand et al. (1971).

Discussion

During the period from 69 to 76 days of gestation, freeze-fracture replicas of both P and E faces show mostly continuous, but partially particulate tight junctions, suggesting structural discontinuity. As noted above, however, permeability measurements carried out on foetal lamb lungs during this time indicate that the epithelium is functionally 'tight' (Olver et al. 1978) and similar to those of more mature lamb foetuses (Normand et al. 1971). This apparent discrepancy between structural and functional observations was resolved by the analysis of complementary double replicas,
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which revealed that many of the discontinuities on the P face are due to a separation, during fracturing, of segments of junctional strands onto the E face. At this stage of development, therefore, epithelial tight junctions are structurally continuous and functionally ‘tight’. This is further supported by the fact that by 69 days, in many of the junctions, the number of strands composing the junction is similar to the value observed at term (Table 2). That the number of strands in a tight junction may play a role in junctional permeability was suggested by a recent study (Claude & Goodenough, 1973) in which a correlation between the number of strands and junctional permeability, in a variety of tissues, was observed. It has been suggested by Mollgard, Malinowska & Saunders (1976), that there may be some exceptions to this generalization. From 76 days of gestation onwards the freeze-fracture appearance of most of the tight junctions of peripheral air spaces is similar to those of full-term lungs. Both morphological and functional evidence, therefore, suggests that ‘tight’ intercellular junctions are established at an early stage of foetal lamb lung development.

At present, due to the small size of the lamb foetus, it is not possible to measure epithelial permeability earlier than at 69 days of gestation. Morphologically, however, epithelial tight junctions at earlier stages of development (39 days) are more tenuous in appearance than in older foetuses. In a number of areas these junctions are composed of one or at the most two rows of particles or strands. In other areas rows of particles appear to be separate from the main network of the junction, giving a quasi-discontinuous appearance to a portion of the junctional network. Finally, most of the junctions are composed of rows of particles or short segments of strands on both P and E faces, rather than a smooth continuous network of strands on the P face and complementary particle-free grooves on the E face. The particulate appearance and the apparent merging or coating of junctional particles to form smooth uninterrupted strands, as well as the initial separation of junctional particles on to both the P and E faces, are features of developing tight junctions in amphibian embryos (Decker & Friend, 1974), chick embryo (Revel, Yip & Chang, 1973), foetal rat liver (Montesano, Friend, Perrelet & Orci, 1975), and foetal kidney of rats and man (Humbert, Montesano, Perrelet & Orci, 1976). It remains to be determined what physico-chemical interactions take place between junctional particles or strands of the same cell, between junctional strands of adjacent cells, or between the strands and the surrounding membrane lipid or underlying cytoplasmic structure to account for the difference in partitioning of junctional particles on to P and E faces during freeze fracturing at different stages of gestation.

Because the lining epithelium of the peripheral air spaces constitutes much the largest surface area interposed between lung liquid on one side and interstitial fluid and plasma on the other, it has been suggested that the entire epithelium participates in the elaboration of lung liquid (Olver & Strang, 1974). The cells lining the air spaces of foetal lamb lungs, up to 109 days’ gestation and before type II pneumocytes can be identified, lack numerous mitochondria, basal infoldings, surface microvilli, or intracellular canaliculi, structures which have been observed in secretory epithelia such as avian salt glands (Ernst & Ellis, 1969), fish gill chloride cells (Philpott & Copeland, 1963) and choroid plexus (Tennyson & Pappas, 1968). Nevertheless some cells in the
foetal lung epithelium are clearly capable of coupling metabolic activity to ion pumps. Furthermore, the epithelium contains long, narrow intercellular channels closed at one end by the tight junctions discussed above. Diamond & Bossert (1967, 1968) have postulated that active ion transport into or out of such lateral spaces produces a standing osmotic gradient within the spaces which then drives water to one or the other side of the epithelial cell layer. It is significant that even in the latter part of gestation, when the lining epithelium is becoming flat, the long intercellular channels are maintained by complex interdigitations of the adjacent cell membranes.

Two additional observations emerged from the present study. The first of these revealed that during the glandular and canalicular stages of development, gap junctions are associated with tight junctions. Such an association between the two types of junctions has been described in other foetal tissues (Decker & Friend, 1974; Revel et al. 1973; Montesano et al. 1975; Humbert et al. 1976). These gap junctions, which electrically couple adjacent cells (Loewenstein & Kanno, 1964) and permit metabolic exchange between them (Gilula, Reeves & Steinbach, 1972), are absent in the alveolar stage, when cellular differentiation is established. That gap junctions may play a diminished role as differentiation progresses is suggested by the observation that during differentiation of ovarian granulosa cells, gap junctions are apparently removed by internalization (Albertini & Anderson, 1974).

The second additional observation was that desmosomes in peripheral pulmonary epithelium were present throughout gestation. These button-like areas of adhesion which securely connect adjacent cells (Kelley, 1966), promoting mechanical stability, are absent from adult, mammalian alveolar epithelium (Schneeberger et al. 1968). In the epithelium of foetal air spaces they may be important in cross-linking adjacent cells at a time when the developing tight junctions are not mechanically strong. In addition, the presence of an occasional desmosome towards the base of the long intercellular space may serve to maintain the geometrical configuration of this potentially important space.

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