

OSMIOPHILIC LAMELLATED BODIES AND ASSOCIATED MATERIAL IN LUNG ALVEOLAR SPACES

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The presence of osmiophilic, lamellated bodies in cells of the pulmonary surface epithelium of both immature and mature animals now is established. Such inclusions were described first by Schlipkötter (1954), and there have been numerous reports of their presence in the lungs of many mammals and in the gill epithelium of amphibia and fish (e.g., Campiche, 1960; Policard et al., 1957). There have been suggestions that they are transformations of mitochondria (Woodside and Dalton, 1958; Schulz, 1959) and that similar bodies "in specific (epithelial) cells" may be observed occasionally to burst and discharge their osmiophilic content into the alveolar spaces (Kisch, 1960). Buckingham and Avery (1962) and Klaus et al. (1962) have proposed that these bodies may be the source of the "surface active agent," the presence of which in the fluid film lining the pulmonary alveoli is now generally accepted (Clements, 1957, 1962; Pattle, 1958). This material is believed to function as an anti-atelectasis factor, in that not only is it capable of changing surface tension but it also can achieve a low tension. The material probably is a lipoprotein, perhaps containing dipalmitoyl lecithin (Clements, 1962). Surface-active agent (surfactant) is present also in the newborn as well as the adult, and osmiophilic inclusion bodies in pulmonary epithelium have been reported in embryonic, neonate, and adult mice (Woodside and Dalton, 1958) and rats (Leeson and Leeson, 1964). Thus, it is possible to postulate a chain of events whereby the osmiophilic, lamellated inclusion bodies in pulmonary surface epithelium, perhaps derived from mitochondria, discharge a lipoprotein into the alveolar

spaces where it acts as a surface-active agent in the fluid film lining the alveoli.

MATERIALS AND METHODS

Small pieces of lung tissue were obtained from adult male and female rats and from neonate rats delivered by Caesarian section on the estimated day of parturition. Some of the neonates from each of two litters were sacrificed before breathing; others were delivered and permitted to breathe for 5 min before killing by decapitation. Tissue was fixed either in 1% osmium tetroxide buffered to pH 7.4 with Veronal-acetate or in 1.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.20 at 4°C for periods of 0.5 to 12 hr. The glutaraldehyde-fixed material was postfixed in osmium tetroxide for 2 hr after washing in sucrose buffer. After dehydration, all material was infiltrated with and embedded in Maraglas (Freeman and Spurlock, 1962). Various staining methods and combinations were employed, usually lead hydroxide (Watson, 1958) alone or in combination with 1% potassium permanganate. Grids were examined in a Philips EM200 microscope. Enzymatic digestion with lecithinases C and D was attempted, both on the sections and on small tissue blocks before processing.

OBSERVATIONS

In many alveolar spaces, particularly in close proximity to pulmonary surface epithelium in nondistended alveoli (Fig. 4), and in a few terminal bronchioles in relation to cilia (Fig. 1), there are collections of small osmiophilic bodies showing a lamellated appearance identical with that shown by the lamellated inclusion bodies of the pulmonary surface epithelium. Associated with the bodies in the alveolar spaces are occasional cell

debris and collections of material in irregular, gridlike patterns (Figs. 2 to 5). These masses of material vary greatly in size, are not delineated by a membrane, and always are related closely to the osmiophilic, lamellated bodies. The lamellated bodies found free in alveolar spaces are not always associated with the membranous material, however.

The pattern of membranes in these masses varies greatly but usually is in the form of parallel membranes in straight lines and concentric curves (Fig. 3). Square grid patterns also are seen commonly, and a small central nodule may be present in the center of each square. At higher resolution, there possibly appears to be continuity of these membranes with those of the lamellated bodies (Figs. 3, 5, 7). The membranes are approximately 27 to 30 A in diameter, with a center-to-center spacing of about 260 to 280 A. At high resolution they appear to have a granular composition, with some fine granular material in the electron-lucid spaces between them (Fig. 3). Lecithinase treatment of sections or of tissue before fixation appears to have no effect on their appearance (Fig. 7), although they perhaps are reduced slightly in bulk.

DISCUSSION

The appearance of this membranous material certainly is reminiscent of phospholipid artificial membranes (Stoeckenius, 1962; Fernández-Morán, 1962). The lamellated inclusion bodies of the pulmonary surface epithelium presumably are lipoprotein in nature. While their origin remains in doubt, there is some evidence that they are released into the alveolar spaces although in this investigation and a preceding study (Leeson and

Leeson, 1964) only one section supported this view (Fig. 6). However, they are present within the pulmonary surface epithelium for some 3 days before birth in the rat whereas they are found free in alveoli only from the day of birth. There is, therefore, a time relationship suggesting that they are released into alveoli; and, indeed, they occasionally are seen in terminal bronchioles also.

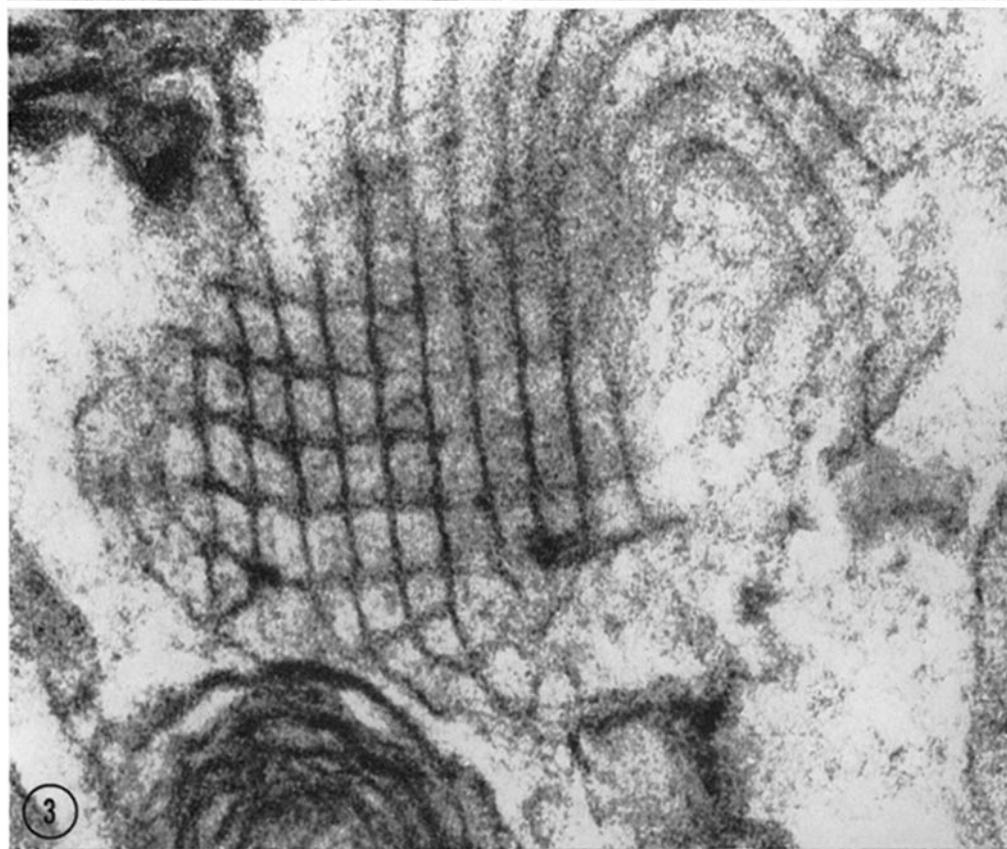
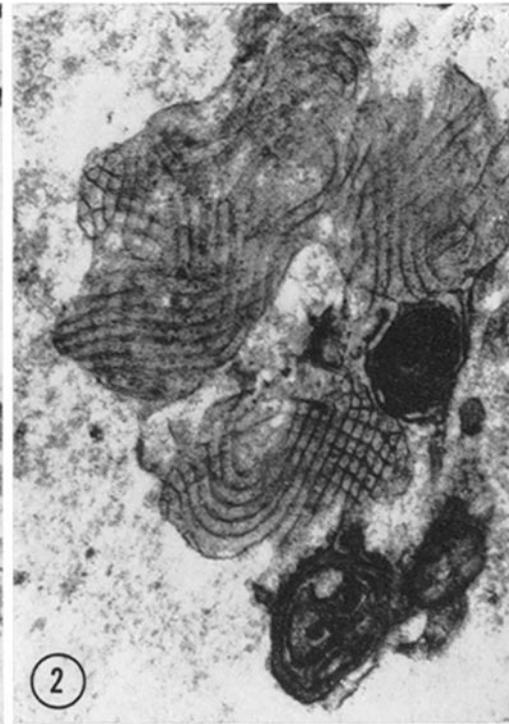
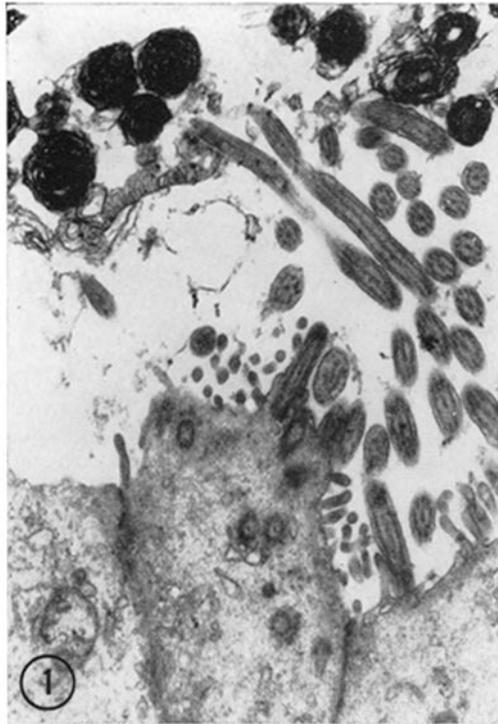
Some micrographs certainly are suggestive of a possible continuity of the gridlike material with the membranes of the lamellated bodies. However, it is not possible to eliminate overlap artifacts. A possible continuity may indicate that the material is derived from the lamellated bodies, but the relationship, of course, may be secondary. The failure of digestion of the membranous material by lecithinases C and D would indicate that the material does not consist only of a lecithin, if the digestion were such as to permit enzymatic action to occur. While it was anticipated that enzymatic digestion on sections of plastic-embedded tissues would not be successful, the lack of an effect on tissue before fixation is notable. The continuity of the membranous material with the membranes of the lamellated bodies, the diameter and spacing (260 to 280 A) and gridlike patterns of the membranes, and the absence of axial periodicity eliminate the possibility that this material is fibrin (periodicity of 230 A, diameter of 100 A: Levene, 1955; Hall, 1963).

The relation of the membranous material to the surface-active agent is doubtful. In this investigation the material was not found in fetuses before birth but was present in rats on the day of birth, both in those permitted to breathe and in those delivered by Caesarian section but sacrificed before breathing. Presumably, the surface-active agent has an important role to play in aeration of

FIGURE 1 Neonate rat, 5 min air breathing. The figure shows apical portions of two ciliated cells from a terminal bronchiole and also some dense, lamellated, osmiophilic bodies free in the lumen and closely related to cilia. OsO₄ fixation. × 17,500.

FIGURE 2 Adult rat. A mass of membranous material in parallel array and in gridlike array, closely associated and continuous with the membranes of two osmiophilic lamellated bodies. OsO₄ fixation. × 62,000.

FIGURE 3 Adult rat. High power view of the membranes shown in a portion of Fig. 2. The possible continuity between these membranes and those of the two lamellated bodies is shown. × 220,000.



the lungs at birth, and thus the time relationship is suggestive that there is some relation between this membranous material and the surfactant.

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FIGURE 4 Neonate rat, no air breathing. Osmiophilic lamellated bodies with associated membranes are shown to be free in an alveolus. A portion of a pulmonary surface epithelial cell appears at bottom of micrograph. Glutaraldehyde-OsO₄ fixation. × 23,500.

FIGURE 5 Higher power view of Fig. 4. It is possible that a continuity exists between these membranes and those of the lamellated bodies. × 82,000.

FIGURE 6 Neonate rat, 5 min air breathing. Apical portion of a pulmonary surface epithelial cell, showing extrusion of an osmiophilic lamellated body. OsO₄ fixation. × 56,000.

FIGURE 7 Adult rat. Membranes in a grid pattern associated with lamellated bodies, after treatment of fresh tissue for 1 hr with lecithinase C. Glutaraldehyde-OsO₄ fixation, × 115,000.

