observations of other cases of this disease will probably provide a better understanding of the significance of nucelar bodies in this pathological condition.

Riassunto. Nel corso dello studio ultrastrutturale di una biopsia renale di un paziente con sindrome di Alport sono stati frequentemente osservati corpi nucleolari nelle cellule endoteliali glomerulari. Gli autori descrivono tale reperto e ne discutono il possibile significato.

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Electron Microscopy of the Pulmonary Alveolar Cells (Granular Pneumocytes) of Normal and Vagotomized Amphibian (*Bufo icterus icterus*)

The relationship between mammalian foetal lung surfactant production and the lamellar osmiophilic inclusions in granular pneumocytes (pneumocytes, epycites, niche cells, wall cells, septal cells, type II alveolar cells) has been extensively studied[1-4].

MILLER and BONDURANT[5] have compared the surface-active properties of lung extracts prepared from amphibians, reptiles, birds and mammals, and conclude that a distinctive surface-active material is limited only to mammalian lungs. Similar conclusions were made by CLEMENTS[6], who found no surface-active agents in the lung of the frog. Although non-mammalian lung extracts do not have surface tension-lowering activity, significant amounts of palmitoyl and lecithin can be found in these preparations[7]. The so-called osmiophilic inclusions, related to surfactant production in mammals, are present

[1] M. KLAUS, O. K. REISS, W. H. TOOLEY, C. PIEL and J. A. CLEMENTS, Science *137*, 750 (1962).
[2] S. BUCKINGHAM, W. F. MCNARY JR. and S. C. SOMMERS, Science *145*, 1192 (1964).
[3] J. KIKKAWA, E. K. MOTOYAMA and L. GLUCK, Am. J. Path. *52*, 177 (1968).
[4] S. BUCKINGHAM and M. E. AVERY, Nature, Lond. *193*, 4815 (1962).
[5] D. A. MILLER and S. BONDURANT, J. appl. Physiol. *16*, 1075 (1961).
[6] J. A. CLEMENTS, Scient. Am. *207*, 121 (1962).
[7] W. R. HARLAN JR., J. M. MARGRAF and S. I. SAID, Am. J. Physiol. *211*, 855 (1966).

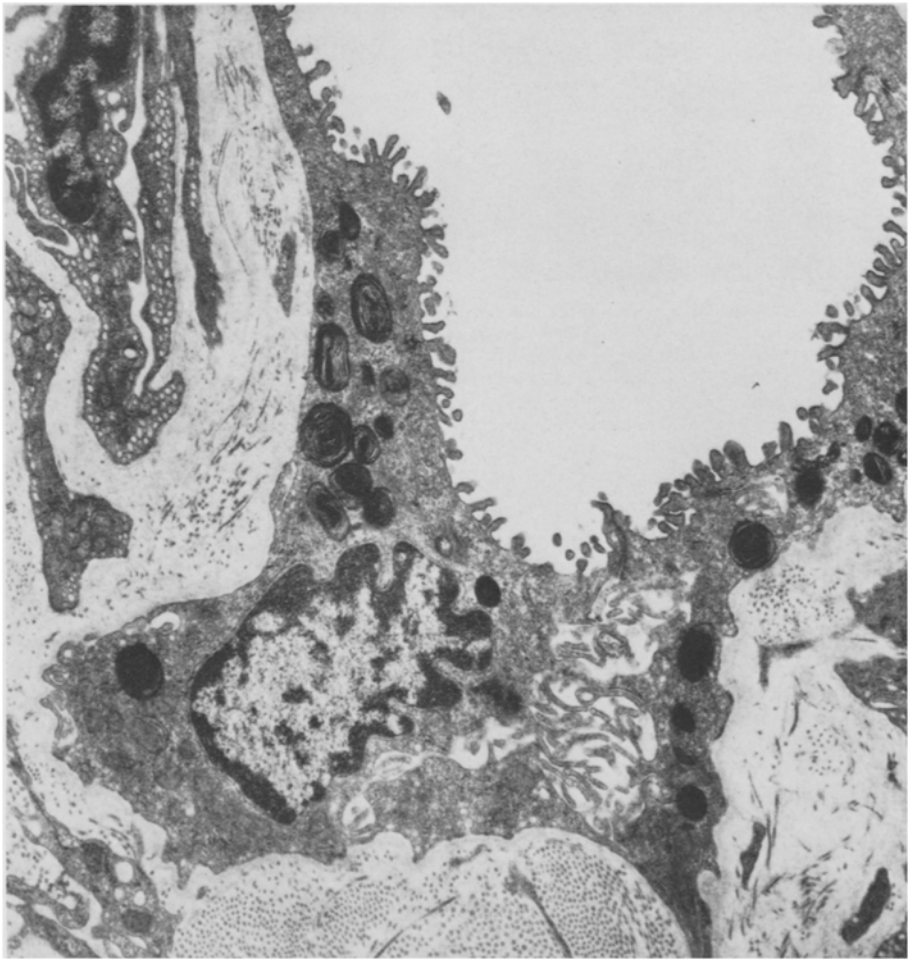

Fig. 1. Electron micrograph of the toad lung showing one type of alveolar cell (granular pneumocyte) with large and clear nucleus, abundant lamellar osmiophilic inclusions and microvilli protruding into the alveolar air space ×10,300.

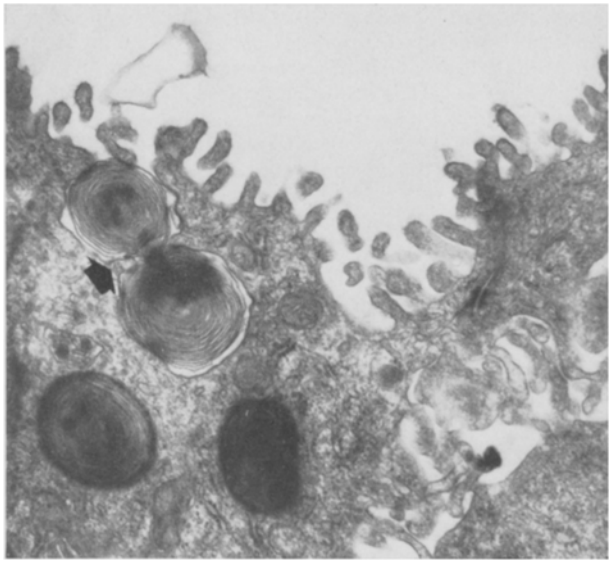

Fig. 2. The arrow points large lamellar osmiophilic inclusions at the vicinity of cell appex which are being ejected. Several microvilli are present in alveolar lumen. ×20,500.

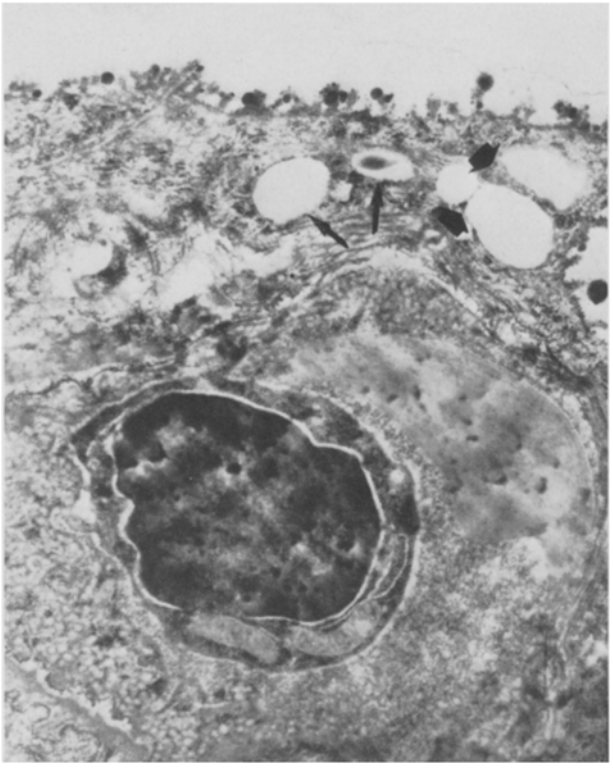

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Fig. 3. Granular pneumocyte extracted with chloroform-methanol. The most part of the inclusions material has been extracted (arrows). ×15,000.

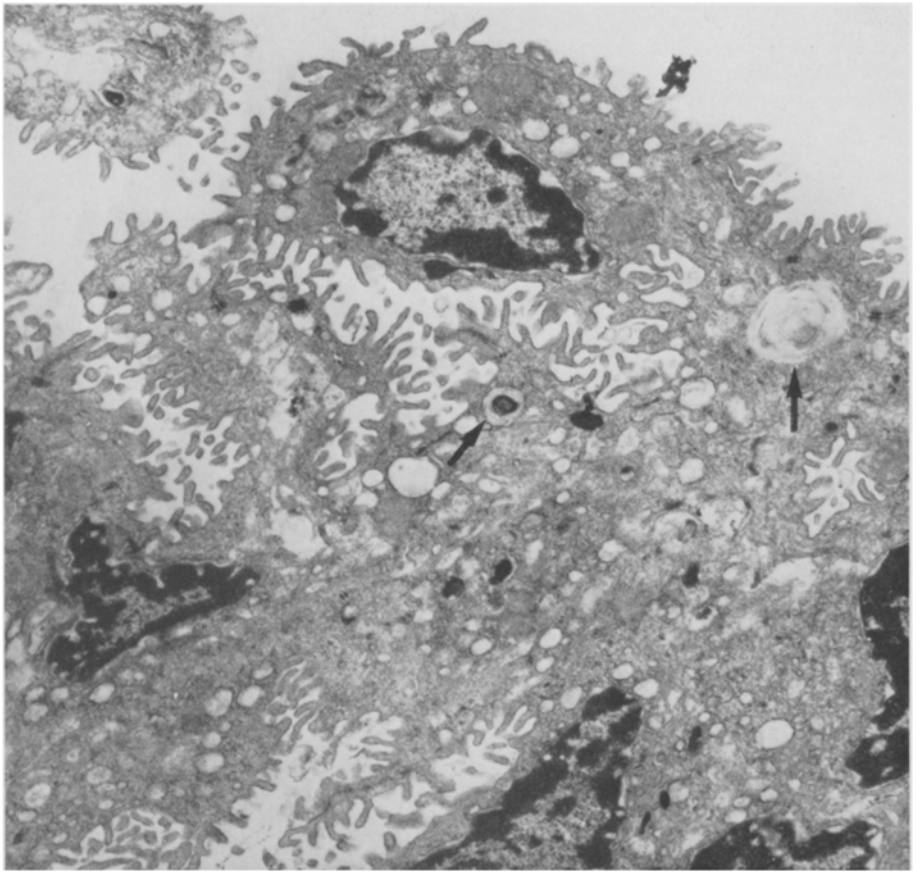

Fig. 4. Granular pneumocyte from lungs of 36 h post vagotomized toad with loosening of lamellar osmiophilic inclusion (arrows). ×10,300.

in large amounts in the granular pneumocytes from amphibian lungs, as reported by BARGMAN and KNOOP[8].

It has been established that vagotomy in mammals[1,9] alters the surface tension properties of lung extracts and induces an almost complete disappearance of the lamellar osmiophilic inclusions from granular pneumocytes. As the lung of the frog seems not to produce surface active agents similar to those found in mammals, and large amounts of osmiophilic inclusions can be observed in the granular pneumocytes of this species, it was decided to investigate their basic chemical composition and whether these structures are under vagal control.

Material and methods. 6 (male adult) toads (*Bufo icterus icterus*) were used for this experiment. 4 of these animals were bilaterally vagotomized, under ether anesthesia, at the mid cervical region. The frogs were maintained in normal laboratory conditions and their lungs removed immediately after natural death. The lungs removed from two untreated animals were used as control.

Fragments of lung fixed for 2 h in 2% glutaral-dehyde in 0.32 *M* phosphate buffer (pH 7.2) at ice temperature followed by fixation in 1% OsO_4 for 1.5 h, were dehydrated in ethanol and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and studied with a Zeiss EM9S2 electron microscope at 60 K_v. Fragments of lungs removed from control animals were fixed as above, immersed for 5 h in 2 ml of 2:1 chloroform-methanol mixture[10] at 60 °C and embedded in araldite.

Results and conclusions. Only one type of alveolar cells could be found in the normal lung of the frog (Figure 1). These cells displayed abundant lamellar and osmiophilic cytoplasmatic inclusions, characteristic of the granular pneumocytes[11]. Occasionally, the spatial relation between the cell membrane and these inclusions strongly suggests that the latter are being extruded into the alveolar lumen (Figure 2). The treatment of normal lung fragments by the chloroform-methanol mixture, determined the disappearance of the lamellar osmiophilic inclusions (Figure 3).

The vagotomized animals died in a period of 4–36 h after surgery. The most consistent and notable alterations in the ultrastructure of the lungs were those related to the morphology of the granular pneumocytes. 36 h post vagotomy, a marked attenuation of the lamellar inclusions, as well as a decrease in their osmiophilic, was observed. The analysis of these cells have also shown an almost complete absence of mitochondria (Figure 4).

We suppose it is permitted to conclude that: 1. The alveolar wall of the normal lung of the frog is lined by only one cell type i.e., by granular pneumocytes. 2. The lamellar osmiophilic inclusions observed in the cytoplasm of these cells seems to have a similar composition to those in mammalian pneumocytes[10]. 3. The almost complete absence of the osmiophilic granular inclusions after vagotomy is in accordance to conditions in mammalian lungs[1,9].

These findings support the hypothesis that the production of the lamellar osmiophilic inclusions in the granular pneumocytes from the lung of the toad, in a similar way as in mammals, is under vagal control. However, we suppose it is licit to speculate about the real relation between the granular pneumocytes and surfactant production. If no lowering of tension activity could be demonstrated in the lung of the frog, what may be the participation of the osmiophilic inclusions in lung physiology and pathology?

Zusammenfassung. Nachweis einer einzigen Zellart vom Typ der granulären Pneumocyten in der Alveolarwand normaler Froschlungen. Die osmiophilen Zelleinschlüsse (Chloroform-Methanol-löslich) verschwinden 36 h nach Vagotomie wie bei Säugetieren.

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[8] W. BARGMANN and A. KNOOP, Z. Zellforsch. *44*, 263 (1956).
[9] V. E. GOLDENBERG, S. BUCKINGHAM and S. C. SOMMERS, Lab. Invest. *16*, 693 (1967).
[10] F. B. ASKIN and C. KUHN, Lab. Invest. *25*, 260 (1971).
[11] C. C. MACKLIN, Lancet *1*, 1099 (1954).

Innervation des Tracheenblasenepithels bei der Büschelmücke *Corethra plumicornis* (Chaoborus)

Die Larvenformen der Büschelmücke *Corethra* besitzen im Thoralkalsegment und im 7. Abdominalsegment jeweils 2 als Schwebeorgane dienende Tracheenblasen, denen Pigmentzellen aufgelagert sind. Die Form- und Lageveränderung dieser amöboid beweglichen Zellen ist als eine Sonderform des physiologischen Farbwechsels bekannt[1]: auf hellem Untergrund sind die Farbzellen kugelförmig geballt und auf eng begrenzte Areale konzentriert; auf dunklem Untergrund erfolgt eine grossflächige Ausbreitung der Pigmentzellen, bei gleichzeitiger Dispersion des Pigments, die zu einer fast völligen Abschirmung der Tracheenblasen führen kann. Schnürungs-, Implantations- und Injektionsversuche lassen eine Steuerung der Pigmentbewegung durch neurohormonale Faktoren aus den Kopf- und Abdominalganglien[1–3] und durch nervöse Beeinflussung[4] vermuten.

Im Rahmen einer Untersuchung zur Motilität der Farbzellen wurden licht- und elektronenmikroskopische Befunde gewonnen, die auf eine enge morphologische und funktionelle Beziehung des Nervensystems zu den Farbzellen hinweisen.

Material und Methode. Für die Untersuchungen wurden Tiere im 4. Larvenstadium verwendet. Für die Lichtmikroskopie erfolgte die Fixierung der Tracheenblasen in alkoholischer Bouinlösung (Rossmann). Nach der Fixierung wurden sie der Silberimprägnation nach Palmgren unterworfen.

Die elektronenmikroskopischen Präparate kamen nach der Fixierung in Osmiumsäure (2%), gepuffert in Veronalacetat ($5{,}7 \times 10^{-3}$ *M*; pH 7,4) mit 3% Saccarose-

[1] A. KOPENEC, Z. vergl. Physiol. *31*, 490 (1949).
[2] M. DUPONT-RAABE, C. r. Acad. Sci., Paris *228*, 130 (1949).
[3] M. GERSCH, Z. vergl. Physiol. *39*, 190 (1956).
[4] E. HADORN und G. FRIZZI, Revue suisse Zool. *56*, 306 (1949).