Zn, Cu and Mn levels[a] following fluoride treatment

Element	Organ	Control	Group I (10 ppm F)	Group II (25 ppm F)
Zn	Liver	202.6 ±21.73	190.5±28.5	143.7±15.3[c]
Zn	Kidney	168.4 ±17.8	176.8±22.9	226.1±21.2[c]
Zn	Bone (femur)	355.5 ±29.7	373.6±49.7	309.8±30.6[c]
Cu	Liver	22.4 ± 2.17	23.4± 3.83	17.8± 3[b]
Cu	Kidney	20.2 ± 2.3	22.8± 2.8	22.3± 3.1
Cu	Bone (femur)	8.8 ± 1.31	9.2± 1.83	5.17± 1.12[c]
Mn	Liver	22.57± 2.23	22.23± 3.43	17.1± 3.31[c]
Mn	Kidney	18.9 ± 2.61	21.23± 2.87	14.1± 1.8[c]
Mn	Bone (femur)	10.87± 1.37	11.93± 2.1	15.01± 2.6[c]

[a] Values expressed as ppm dry weight tissue (mean ± SD). [b] Significantly different from control, $p < 0.05$. [c] Significantly different from control, $p < 0.005$.

overall metabolism. Copper depletion is known to affect not only the transport of iron but also its utilization for the synthesis of hemoglobin[9]; anemia is reported to be frequent following excessive fluoride ingestion over prolonged periods[10]. Copper deficiency is also known to impair collagen metabolism[9] and so does excessive fluoridation[11]. The alteration in the levels of copper (depletion) and manganese (elevation) are most pronounced in the bone, perhaps due to its high affinity for fluoride[12].

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Origin and development of neuroepithelial bodies in fetal rabbit lungs

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Summary. Light-microscope studies concerning the embryological development of fetal rabbit lungs revealed the occurrence of argyrophilic neuroepithelial bodies in an early gestational stage (i.e. already in the glandular period and from the 18th day onwards). Their morphological characteristics and further differentiation towards birth are detailed.

Neuroepithelial bodies (NEBs) are corpuscles of innervated endocrine-like cells, which we have recently identified in the intrapulmonary airway epithelium of man[2,3] and several mammals[4,5]. They have also been observed in lower vertebrates[6]. Electron microscopically the corpuscular cells are granulated, containing 2 types of dense cored vesicles, of which the first type exhibits a positive reaction for serotonin as demonstrated cytochemically and with fluorescent techniques[4,5,7]. The occurrence of an intracytoplasmic polypeptide substance has been demonstrated by a fluorescamine-induced fluorescence[8]. Also a bombesin-like immunoreactivity was reported in single and grouped cells in bronchial and bronchiolar epithelium of man[9].

The stored substances, the distinct innervation and the reactions of the NEBs to hypoxia suggest that these corpuscles may be intrapulmonary neuroreceptor organs modulated by the central nervous system[4,10-12] and exhibiting local secretory activities.

Earlier studies included mainly mature newborn and occasionally adult animals. Although several investigations incidentally mention numerous NEBs in late fetal lungs[4,13,14], it appeared interesting to know at which precise fetal age NEBs are first detected and how they develop and mature during pulmonary embryogenesis.

Material and methods. 26 pregnant rabbits were i.v. anesthetized with sodium pentobarbital (Nembutal). The date of mating was considered day 0 of gestation; full gestation time for rabbits is 31 days. 195 fetuses were delivered by hysterectomy at regular intervals from day 15 till day 30 of gestation. The fetal lungs were removed for various investigations; 50 representative specimens were immediately fixed in Bouin's fluid, embedded in paraffin, cut in serial

sections and stained with hematoxylin-eosin (H.E.). Argyrophilia was detected according to Bodian's silver proteinate technique as modified by Van Campenhout[15] and by Grimelius' silver nitrate technique[16].

Results. The results obtained are somewhat different on H.E. and silver impregnated sections.

On H.E.-stained sections cells grouped into NEBs are first recognized in the lungs of 21-day-old fetuses (fig. 1). They are intercalated within the respiratory mucosa and extend from the basement membrane to the airway lumen. The corpuscular cells are easily seen, exhibiting a dense eosinophilic cytoplasm in contrast to the clear and translucent, glycogen containing lung epithelial cells. In this early fetal period the nuclei of the NEBs are paler, and also more even and uniform than the nuclei of the surrounding pulmonary epithelial cells.

At this stage of lung development, some NEBs form a rather irregularly oriented cell cluster while other NEBs have already differentiated towards a more organized structure. This differentiation rapidly takes place from the hilar area to the peripheral parts of the lung. The differentiated NEBs consist of a row of high and columnar epithelial cells with elongated nuclei, arranged in an orderly way, surrounded by neighbouring cells which cover a small part or the whole apical cell pole of the NEBs. From this moment on the fetal NEBs exhibit a light-optical morphology identical with that seen in mature newborn rabbits. At the end of the glandular period they are quite numerous, occurring within the mucosa of every intrapulmonary airway and being frequently located at bronchiolar bifurcations.

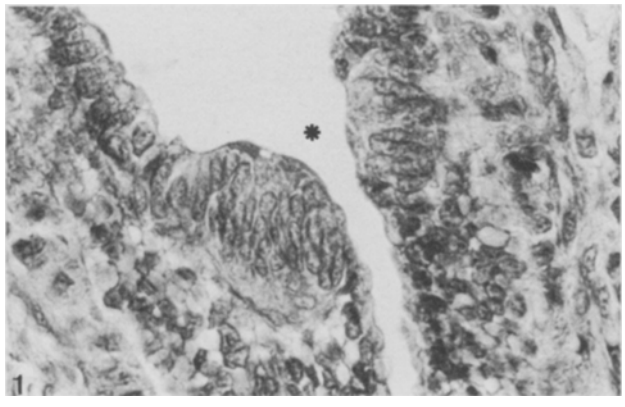

Figure 1. 2 neuroepithelial bodies (*) facing each other and intercalated within the bronchial mucosa, fetal rabbit lung, 21 days' gestation, H.E. × 600.

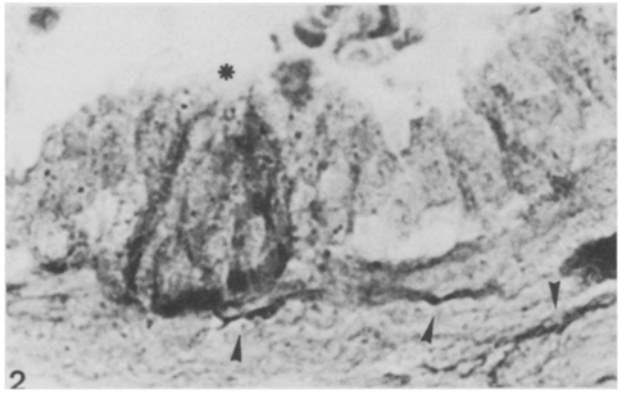

Figure 2. Branching subepithelial bronchial nerve fibers (→), ending upon a characteristic Neuroepithelial Body (*), which reveals a distinct and mainly basal cytoplasmic argyrophilia, fetal rabbit lung, 21 days' gestation, Grimelius' silver nitrate technique, × 1000.

On silver-impregnated sections (fig. 2) NEBs are still earlier and more easily detected. Using Grimelius' technique a cluster of slightly argyrophilic cells intercalated within the epithelium is already observed in the early glandular period (i.e. at the 18th day) with nerve fibers running in close proximity to it. The cytoplasmic argyrophilia becomes more prominent with increasing fetal ages. Nerve fibers which ramify into the NEBs and contact the cylindrical corpuscular cells can be detected from the 19th day of gestation onwards. In 20-day-old fetuses the NEBs are seen in much greater number. At this time also isolated argyrophilic Kultschitzky-like or so-called AFG cells[17,18] which are argyrophilic, fluorescent (after freeze-drying and formaldehyde vapour treatment) and ultrastructurally granulated, are often seen. The NEBs are well developed, some already consisting of about 15 cells.

During the growth of the conducting airways in the ensuing glandular, canalicular and alveolar stages of lung development a parallel development in the number of NEBs is seen. Finally it may be mentioned that while using Bodian's silver proteinate technique the cytoplasmic argyrophilia of the corpuscular cells first becomes visible only from the 25th day onwards.

Discussion. Our light optical study reveals that despite the immature appearance of lung structure, NEBs are already well-differentiated at an early stage of lung development. Recently Hung[19] found slightly argyrophilic NEBs in 21-day-old rabbit fetuses, while our study reveals a slightly argyrophilic cell cluster already at the 18th day and numerous NEBs at 20 days' gestation. Also, the early contact which we observed between nerve fibers and NEBs on day 19 is not mentioned by Hung[19] who describes this association between the nerve fibers and the corpuscular cells of the NEBs at a much later fetal age (30 days' gestation).

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