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Vitamin A and ciliated cells
I. Respiratory epithelia

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Summary: To estimate the role of vitamin A on ciliated cells we investigated whether ciliated cells undergo any alteration during vitamin A deficiency. The epithelia examined include the ciliated cells of the respiratory tract and the ciliated sensory cells of the inner ear, the tongue, and the olfactory cells. This part of the paper will describe the ciliated epithelium of the tracheobronchial tract and its relation to vitamin A status. During vitamin A deficiency a partial loss of ciliae can be observed before any squamous metaplasia (which usually occurs during longer lasting vitamin A deficiency) develops. The scanning electron microscopic data illustrate the altered surface of the epithelium during vitamin A deficiency better than transmission electron microscopy.

Zusammenfassung: Um zu prüfen, in welcher Weise Vitamin A zilientragende Epithelien beeinflußt, wurde untersucht, ob zilientragende Epithelien im Vitamin-A-Mangel besondere Veränderungen aufweisen. Die untersuchten Gewebe beinhalten das Respirationsepithel sowie die zilientragenden Sinnesepithelien des Innenohres, der Zunge und des Riechepithels. Dieser Teil beschreibt die Veränderungen des zilientragenden Epithels des Tracheobronchialtraktes in Relation zum Vitamin-A-Status. Mit rasterelektronenmikroskopischen Methoden konnte im Vitamin-A-Mangel ein fleckiger Verlust von Zilien des Respirationsepithels beobachtet werden, bevor es zu einer squamösen Metaplasie des Gewebes – wie sie im Vitamin-A-Mangel beschrieben ist – kam. Die rasterelektronenmikroskopischen Aufnahmen stellen die Veränderungen an den zilientragenden Zellen dieses Epithels anschaulicher dar, als dies mit transmissionselektronenmikroskopischen Verfahren möglich ist.

Key words: ciliated cells, tracheobronchial tract, scanning electron microscopy, vitamin A deficiency

Introduction

It is argued that ciliated cells contain vitamin A and need it for their function (1). This hypothesis can be strengthened by the fact that in vitamin A deficiency the function and morphology of so-called ciliated cells seems to be affected. Ciliated epithelia include the respiratory epithelium as well as the ciliated area of tuba uterina and ductus deferens. In addition ciliae containing sensory cells belong to this type of ciliated area. With regard to this assumption it is interesting that in vitamin A

Table 1. Mean concentration and relative density of retinyl esters in different organs of the guinea-pig.

Total dry weight (g)		Concentration* µg/g dry weight	Relative density µg/organ
Liver	7.54 ± 1,54	185.10	1395.65
Kidney	2.37 ± 0.39	2.31	5.47
Testicle	1.44 ± 0.18	10.59	15.25
Trachea	0.19 ± 0.05	0.501	0.10
Lung	0.96 ± 0.33	0.983	0.94
Eye	0.17 ± 0.01	26.92	4.68
Tongue	0.49 ± 0.21	3.28	1.60
Inner ear	0.001 ± 0.002	7.30	0.0073

* Mean values

deficiency sensory epithelia and function are affected. The question arises whether there might exist a common pathogenesis of alteration of ciliated cells or epithelia. We therefore decided to test different epithelia with morphological and biochemical methods to come to a better understanding of the underlying process of disorders of ciliated cells in vitamin A deficiency.

As we previously reported (2, 3) ciliated epithelia contain different concentrations of retinyl esters, the storage form of the vitamin. The amount of retinyl esters in these epithelia was very high compared with other non-ciliated areas. In the trachea and lung we detected the following concentrations and distribution (Table 1). In comparison with the liver – the main storage organ – this concentration is not very high but compared with muscle or brain we found high amounts. The detection of retinyl esters in the tracheobronchial epithelia does not reveal the hypothesis that these epithelia need vitamin A for their function. Yet the increased susceptibility of tracheobronchial infections in vitamin A deficiency (4, 5) and the transmission electron microscopic data of several authors (6, 7) which show squamous metaplastic changes of this epithelium during vitamin A deficiency clarifies that the tracheobronchial epithelium needs vitamin A for its function and development. We know that this epithelium undergoes squamous metaplastic changes in vitamin A deficiency but it is not known why an increased susceptibility to tracheobronchial infections exists during moderate deficiency.

The question arises whether the mucociliary clearance system is affected just at the beginning of a vitamin A deficiency, which could clarify the increased susceptibility against infections of the tracheobronchial tract during development of vitamin A deficiency.

Materials and Methods

Animals

Young guinea-pigs (mean weight 540 ± 35 g) were fed a vitamin A free diet over 90 days. The animals were divided into two groups: the normal group which served as

control was supplemented with 100 IE vitamin A/day whereas the other group (deficient animals) was not supplemented. Each group contained six animals.

After 15, 40 and 90 days liver and serum values of vitamin A (retinol, retinyl ester) were controlled by HPLC.

When the serum retinol values had dropped near zero the liver stores contained at least 7 IE vitamin A. At this timepoint there were no signs of severe or longer lasting deficiency such as Bitot's spots or conjunctional xerosis. Especially infections of the tracheobronchial tract had not developed. Both groups (normal and deficient) had equal body weight and behaviour prior to the experiment.

Scanning electron microscopy

The animals were anaesthetized (2 mg Nembutal/kg body weight) and perfusion fixed according to Ito and Karnovsky (8). The tracheal tubes were dissected and the lung was prepared. The organ samples were washed in 0.1 M cacodylate buffer (pH 7.3), treated with 1 % osmium tetroxide solution and dehydrated in grades ethanol series and amylacetate. The critical point drying was performed in a Balzers critical-point drying apparatus (M 1202) with CO_2 and amylacetate. Dry specimens were coated with gold in a Polaren Sputter coater and examined in a scanning electron microscope Novoscan 30 (Zeiss, FRG).

Results and Discussion

In normally nourished animals the tracheobronchial epithelium is a heterogenic pseudo stratified epithelium which has been described extensively for the rat (9, 10). The columnar epithelium contains four types of cells: ciliated cells, goblet cells, basal and brush cells. Each ciliated cell contains about 270 cilia and is connected with the other epithelial cells by cellular junctions. The ciliae of the predominating ciliated cells (goblet cells: ciliated cells = 1:4) cover the whole surface of the tracheal epithel so that no other structures are visible (11). For the tracheobronchial epithelium of the guinea-pig less information is available. McDowell (12) describes goblet, ciliated and basal cells, which are arranged in a pseudo

Fig. 1. Ciliated tracheal epithelium of a normal nourished guinea-pig.

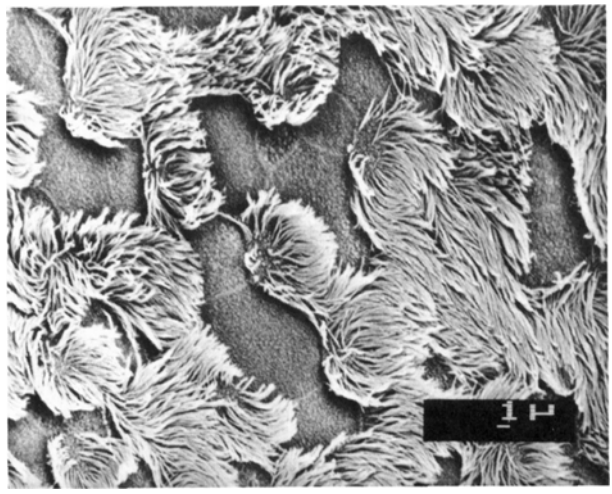


Fig. 2. Tracheal epithelium of a normal nourished guinea-pig with ciliae, microvilli and cell junctions.

stratified form. Some goblet cells overtop the ciliae and cytoplasma protrusions caused by mucous granula can be observed (13). Some cells containing microvilli can be observed between the ciliated cells with a clearly visible network of cell junctions (14).

In our material of the normal nourished group the ciliae of the trachea cover the whole surface (Fig. 1) and single microvilli containing cells with clear visible junctions can be seen (Fig. 2). In contrast, in the deficient

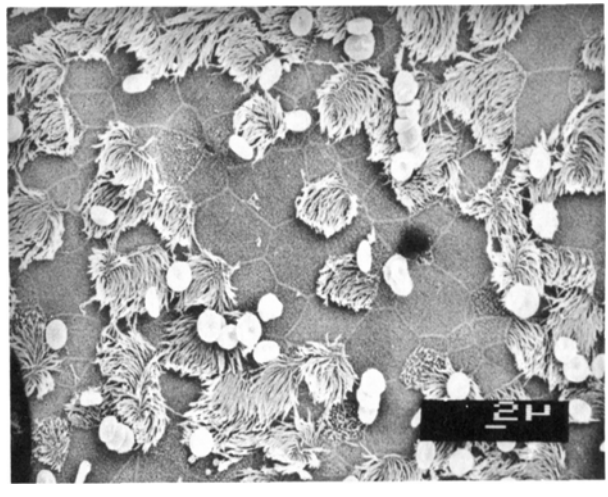
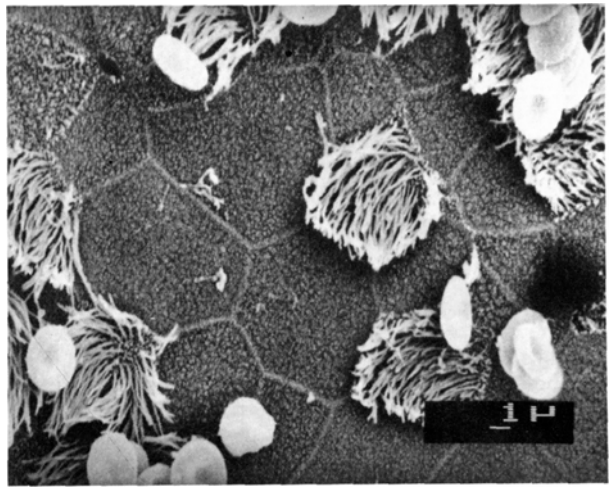


Fig. 3

Fig. 3, Fig. 4. Tracheal epithelium of a vitamin A deficient guinea-pig with partial loss of ciliae and without any signs of squamous metaplastic changes.



group a partial loss of ciliae can be observed (Fig. 3) without any sign of squamous metaplastic changes (Fig. 4). The cells in between the ciliated area show no other signs of alterations visible in the scanning electron microscopic presentation. So it can be assumed that this loss of ciliae is an early sign of a beginning vitamin A deficiency.

Similar alterations of the ciliated cells are detectable in a second order bronchus where the ciliae typically cover the surface in a normal state (Fig. 5) but undergo a partial loss during deficiency (Fig. 6). In contradiction to the ciliated area of the trachea some deciliated cells of the bronchus

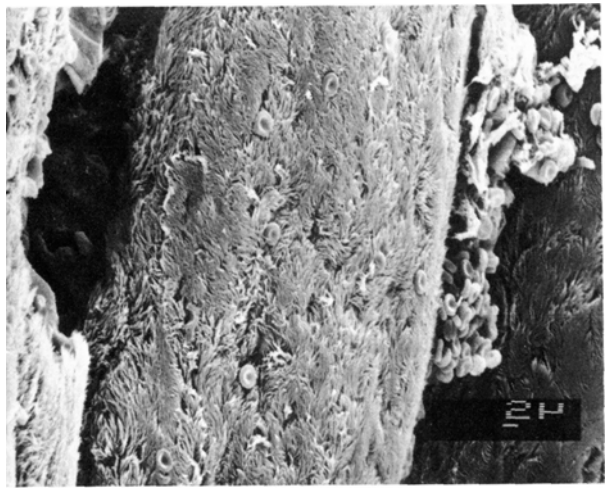


Fig. 4

Fig. 5. Bronchial epithelium (second order bronchus) of a normally nourished guinea-pig.

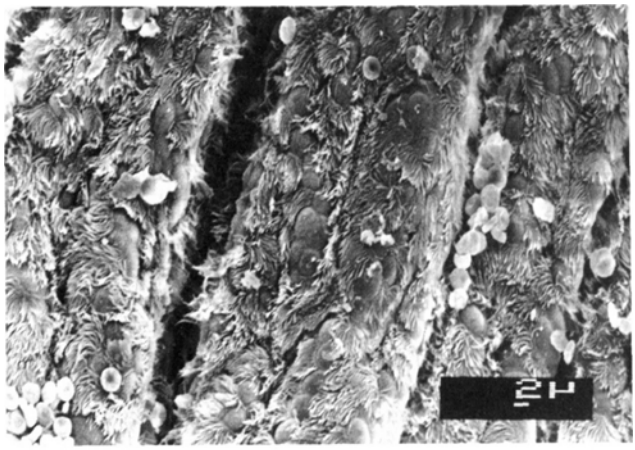


Fig. 6

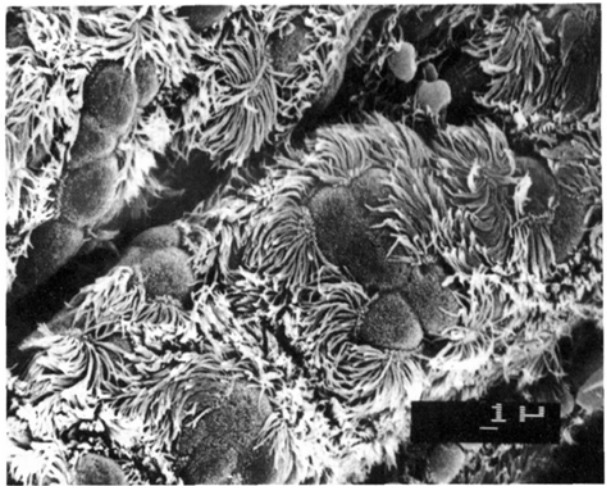


Fig. 7

Fig. 6, Fig. 7. Partial loss of ciliae in a second order bronchial epithelium of a vitamin A deficient guinea-pig. Deciliated cells overtop the remaining ciliated cells.

seem to overtop the remaining ciliae (Fig. 7). It is not clear whether this might be due to basal cell hyperplasia as described during vitamin A deficiency in the tracheobronchial epithelium (14).

Lack of ciliae as a result of squamous metaplasia following vitamin A deficiency has been described with the light- and transmission electron microscopic technique in the past (15, 16, 17). As Boren et al. (18) showed the extent of metaplastic changes of these mucous membranes correlates well with the degree of vitamin A deficiency. The reason for the loss of ciliae in vitamin A deficiency is assumed to be the result of the squamous metaplastic changes caused by the lack of the vitamin (6, 19). Prior to the loss of ciliae the basal cells are altered (17, 18) and start to undergo metaplastic changes as an early sign of vitamin A deficiency (12, 16). Due

to longer lasting deficiency the mucus producing cells start to produce keratin as a sign of squamous metaplasia (16, 17, 20).

Metaplastic alterations and lack of ciliae can be observed in many cases of pulmonary and trachael diseases. Squamous metaplasia with loss of cilia is a common finding in non-neoplastic diseases of the lung (21) and it has been considered that it will act as a stage in tumorogenesis of squamous cell carcinoma (21, 22). Tumor induction in the trachael epithelium by benzo(a)pyrene ferric oxide results in hyperplasia of basal and mucous cells (17, 23, 24) whereas the lesions following vitamin A deprivation are primary produced as a result of hyperplasia of basal cells.

The cellular action and the involvement of vitamin A on metaplastic changes are still a matter of debate. It is suggested that vitamin A acts on RNA metabolism in tracheal epithelium in that an abnormal electrophoretic pattern of the high molecular weight RNA molecules, synthesized in the tracheal epithelium of vitamin A deficient hamsters (25, 26) was observed.

Addition of vitamin A to organ cultures with squamous metaplastic changes caused by vitamin A deprivation results in a reversal of the keratinizing process and replacement of the squamous cells by columnar-ciliated and mucous cells (12, 25). During the process of supplementation of the vitamin in in vitro cultures the cilioneogenesis is induced by vitamin A. Addition of 13 cis retinol to tracheal organ cultures of hamsters leeds to an increased cilioneogenesis whereas citral (3,7-dimethyl-2,6-octadienal) addition is capable to block this cilioneogenesis (27, 28). Furthermore, vitamin A can induce cilioneogenesis and production of mucus in cell culture which typically produce keratin (26).

Squamous metaplasia and lack of cilia is not only seen during tumorogenesis and vitamin A deficiency but also after treatment with other agents such as smoking (21), chronic (29) and acute infections (30) of the bronchial and upper respiratory tract (31, 32, 33) as well as during infections of the nasal tract (34, 35). If we consider the fact that vitamin A can induce the cilioneogenesis and that as an early sign of vitamin A deficiency a partial loss of cilia occurs the question arises whether during noxic induced loss of cilia vitamin A may play a protective role.

With respect to the fact that partial loss of cilia decreases the mucociliary clearance activity of the respiratory epithelium this can be a cause for the increased susceptibility to tracheobronchial infections during vitamin A deficiency. The loss of cilia occurs as the first alteration of the tracheobronchial epithel in vitamin A deficiency before any squamous metaplasia develops. We do not know the time delay between loss of cilia and beginning of squamous metaplasia but this should be revealed in the future.

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