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Morphology and changes in Clara cells in the foetal bronchioles of Swiss mice

A. Blanco, A. Méndez, L. Carrasco, Mª.J. Bautista and M.A. Sierra
Department of Anatomy and Pathological Anatomy, Faculty of Veterinary, University of Córdoba, Córdoba, Spain

Summary. In this work we have studied the morphology and evolution of Clara cells in the bronchiolar mucosa of lungs from 63 Swiss mice foetuses that were classified into three groups according to age (14, 16 and 18 days). A control group composed of 21 15-day-old Swiss mice was also studied.

The most salient feature of the Clara cells observed was the occurrence of two types of secretory granules and a large smooth endoplasmic reticulum . On the other hand, the Clara cells of the control group had a single secretory granule.

Clara cells thus seem to take part in bronchiolar metabolism, as they were quite abundant in the early foetal groups and diminished as birth approached. This cell decrease was confirmed by the control group (15-day-old mice), the bronchioles of which contained scant cells and numerous ciliated cells.

Key words: Clara cells, Lung, Foetal Swiss mice, Morphology

Introduction

Ever since Clara (1937) studied the cells discovered by Kölliker in bronchiolar epithelium in 1881, morphological and functional knowledge about Clara cells has continuously increased. Massaro et al. (1981), who studied rat bronchioles, found clara cells to occur in much lower proportions than ciliated cells (3% vs 97%).

By using a light microscope and applying immunological techniques to protein fraction sera from Clara cells, Strum et al. (1990) found increased numbers of this type of cell in hamster bronchioles that diminished a few days after birth. Several other authors have also reported increased numbers of Clara cells in connection with pathological processes, particularly of toxic nature [e.g. toxic bronchitis in the horse, as reported by Drommer et al. (1987)].

Offprint requests to: Dr. A. Blanco Rodríguez, Departamento de Anatomía y Anatomía Patológica Comparadas, Facultad de Veterinaria, Universidad de Córdoba, Córdoba, Spain

The findings of Etherton et al. (1973) in relation to the synthesis of surfactants in terminal bronchioles, and those of Kuhn et al. (1974), who reported the formation of secretory granules by Clara cells, confirmed their apocrine function. All reports in this context [e.g. that of Plopper et al. (1980) on various species including hamsters, guinea pigs and mice, and Kaup et al. (1985) on horse bronchioles] point to a single secretory granule of medium size and a high electron density lying at the cell apex, close to the cytoplasmic membrane.

The occurrence in these cells of abundant smooth endoplasmic reticulum, which was detected by Drommer et al. (1987) in the horse and Christensen et al. (1987) in the hamster, arises from their ability to store cytochrome P-450 isoenzymes in their microsomal fractions, which take part in the biotransformation of xenobiotic substances. This role of Clara cells was confirmed by Richards et al. (1990), who studied the *in vivo* degradation of pneumotoxic substances by these cells. However, as pointed out by Kanekal et al. (1990), biotransformation processes, whether resulting from direct action of oxidative enzymes or from the occurrence of reaction intermediates, may cause severe cell alterations; the same authors indicate that xenobiotic substances reach Clara cells through both exogenous and blood-borne means.

Clara cells synthesize other substances such as mucous substrates containing abundant proteins - particularly CC 10 - as found by Hagen et al. (1990) in rabbit lung, as well as the above-mentioned surfactants; the occurrence of which was confirmed by Patton et al. (1991), who provided evidence of a positive reaction of type-II pneumocyte granules - those devoted to surfactant storage - in sera from Clara cell fractions. However, there is seemingly no reference to the cytoplasmic site where such substances are located or stored in Clara cells.

Materials and methods

In this work we used lungs from 21 Swiss mice foetuses of different ages (14, 16 and 18 days) plus a control group consisting of lungs from 21 Swiss mice of

15 days that were born to 7 different mothers (Table 1).

The experimental animals were Swiss mice that were supplied by Interfauna Ibérica (Barcelona). They were kept under the typically recommended conditions for this type of experiment (ad libitum availability of food and water, controlled temperature and photoperiod, and prevention of coprophagia).

Both the foetuses and the control mice were sacrificed by decapitation and samples were collected from the cranial and caudal lobes of both lungs and were fixed in 3% glutaraldehyde and subsequently postfixed in osmium tetroxide according to Sabattini et al. (1963). The specimens were then cut into semithin sections that were stained with 10% Toluidine Blue for examination under the light microscope, or in ultra-thin sections that were treated with 3% uranyl acetate and 0.3% lead citrate for electron microscopic analysis. The morphometric determination of the proportions of Clara and ciliated cells in the bronchioles was carried out by light microscopy using semithin sections (1 μm thick). All measurements involved 40 bronchioles, of which 20 each were from the cranial and caudal lobe, respectively. The size of small and large secretory granules was determined under the electron microscope. Five randomly-chosen granules of each type of cell were measured. A total of 60 Clara cells (30 from each lobe) were quantified. Measurements were made by means of photographic lenses at a magnification of 7,500.

Table 1. Animals studied.

GROUP	NUMBER OF SPECIMEN	AGE
I	21	14 days of gestation
II	21	16 days of gestation
III	21	18 days of gestation
Control	21	15 days of age

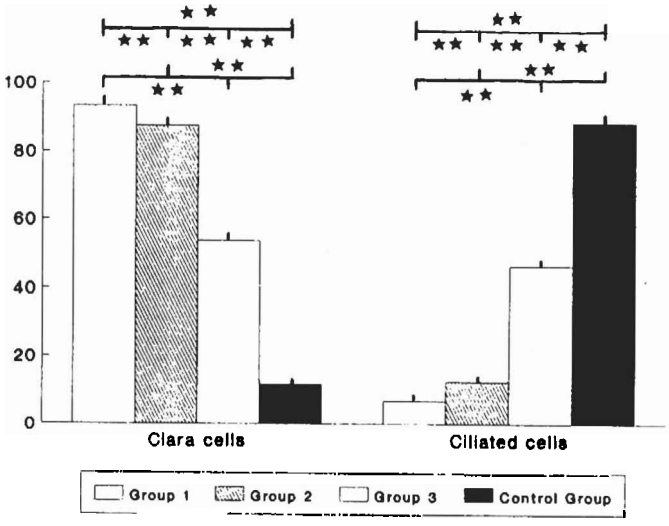

Fig. 1. This morphometric study shows the proportion of Clara cells and ciliated cells in different experimental and control groups. n= 40. **= p< 0.001.

Morphometric analyses, both with the light and the electron microscope, was carried out on an IBAS II morphometer (Interactive Image Analysis System, Kontron).

Results

Bronchioles were readily visible in the lungs from 14-day foetuses and consisted of numerous Clara cells, which accounted for 93.2% of the total (the other 6.8% were ciliated cells) (Fig. 1). The Clara cells were quite prominent in the direction of the bronchiole lumina and their cytoplasm was granulated and deeply stained by basic dyes, in contrast to the ciliated cells, the cytoplasm of which was not stained by basophilic dyes and lacked any granulations (Fig. 3).

The light microscope revealed the occurrence of two types of secretory granules in the cytoplasm: one group - the smaller in number - with a high electron density lying close to the apical region of the cells and measuring 310±18 μm in diameter (Fig. 2) which showed numerous secretory images; and another group, the larger, located in the middle of the cytoplasm and featuring a lower electron density (Figs. 4, 5) and a more widely variable size (429±23 μm) (Fig. 2). In the vicinity of the rough endoplasmic reticulum of the cytoplasm small cisternae were also detected with smooth reticulum that spread throughout and were surrounded by a light cytoplasmic halo in the form of a vacuole (Figs. 4, 5).

On the other hand, ciliated cells contained few cilia (Fig. 4).

The morphological picture of the lungs from Group II foetuses was similar to that described above. However, Clara cells were somewhat less abundant (87.4% vs 12.6% of ciliated cells) (Fig. 6).

By light microscopy, Clara cells were found to extend towards the bronchiolar lumina and to be markedly

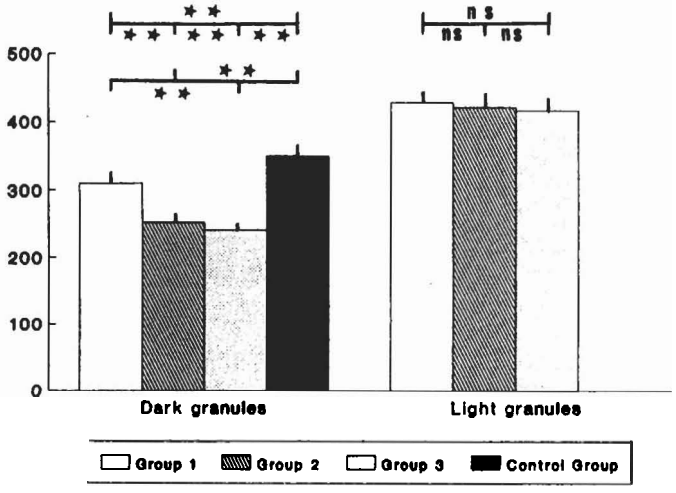

Fig. 2. This drawing shows the proportion of two types of granules found in Clara cells from different experimental and control groups. n= 300. **= p< 0.001. n.s.= no significative difference.

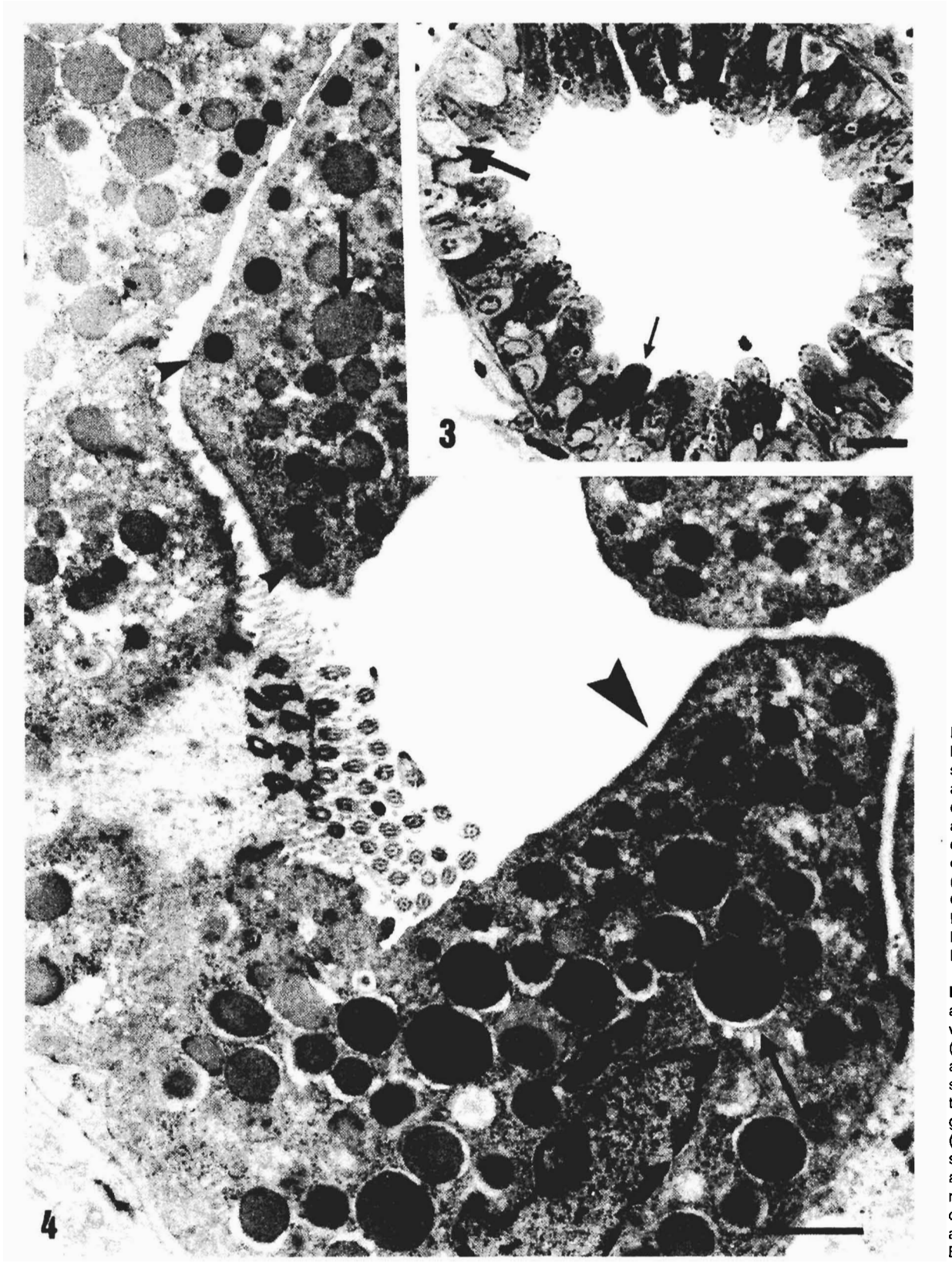

Fig. 3. Bronchiole showing abundant Clara cells (small arrow) and low ciliated epithelial cells (large arrow). Group I. Methylene Blue. Bar= 30 μm.

Fig. 4. Detail of a bronchiole with Clara cells (large arrowhead) showing 2 types of granules (osmiophilic, small arrowhead, and non-osmiophilic, arrow). Group I. Bar = 1 μm.

basophilic. In addition, they showed highly basophilic granules and signs of apical scaling.

Under the electron microscope the two above-described types of granules were observed: a larger group featuring a higher electron density, located at the apical periphery and measuring 253±31 μm in diameter; and a smaller group made up of less dense granules of unchanged location and diameter (422±23 μm) (Fig. 7).

Even though the smooth endoplasmic reticulum preserved its original morphology, it was somewhat more markedly developed and was surrounded by cytoplasmic vacuolations that sometimes contained pseudo-membrane structures. Ciliated cells also preserved their apparently normal morphology (Fig. 7).

The lungs from Group III foetuses were markedly different from those of the other two groups with regards to their cell composition. Thus, the bronchioles consisted of 53.7% Clara cells and 46.3% ciliated cells (Fig. 8), i.e. much more similar proportions of the two types. The former lay closest to the bronchiole lumina and were highly basophilic and occurred in various sizes.

The more dense granules were 241±29 μm in diameter (Fig. 9), while the less dense ones occurred in the same numbers and were 417±19 μm in diameter (Fig. 9). The Clara cells in this group preserved their smooth endoplasmic reticula in the same condition, but featured even more marked vacuolated areas, with small vesicles (Fig. 10).

On the other hand, the morphology of the ciliated cells was perfectly normal (Fig. 9).

Control group

This group consisted of 15-day-old Swiss mice. Their bronchioles contained largely ciliated cells (84%) and scarce Clara cells (16%).

The ciliated cells were morphologically normal. On the other hand, the Clara cells had a cytoplasm with a single secretory granule of 350±20 μm that was somewhat polymorphous and featured a high electron density (Fig. 11). Also, the cytoplasm abounded with smooth endoplasmic reticulum in the form of small clusters.

Discussion

The first salient conclusion of this work was the occurrence of two types of secretory granules in Clara cells from the bronchioles of the studied foetuses. While this confirms that Clara cells are of glandular nature, as suggested by Clara (1937) and later confirmed by a number of authors including Kuhn et al. (1974) in

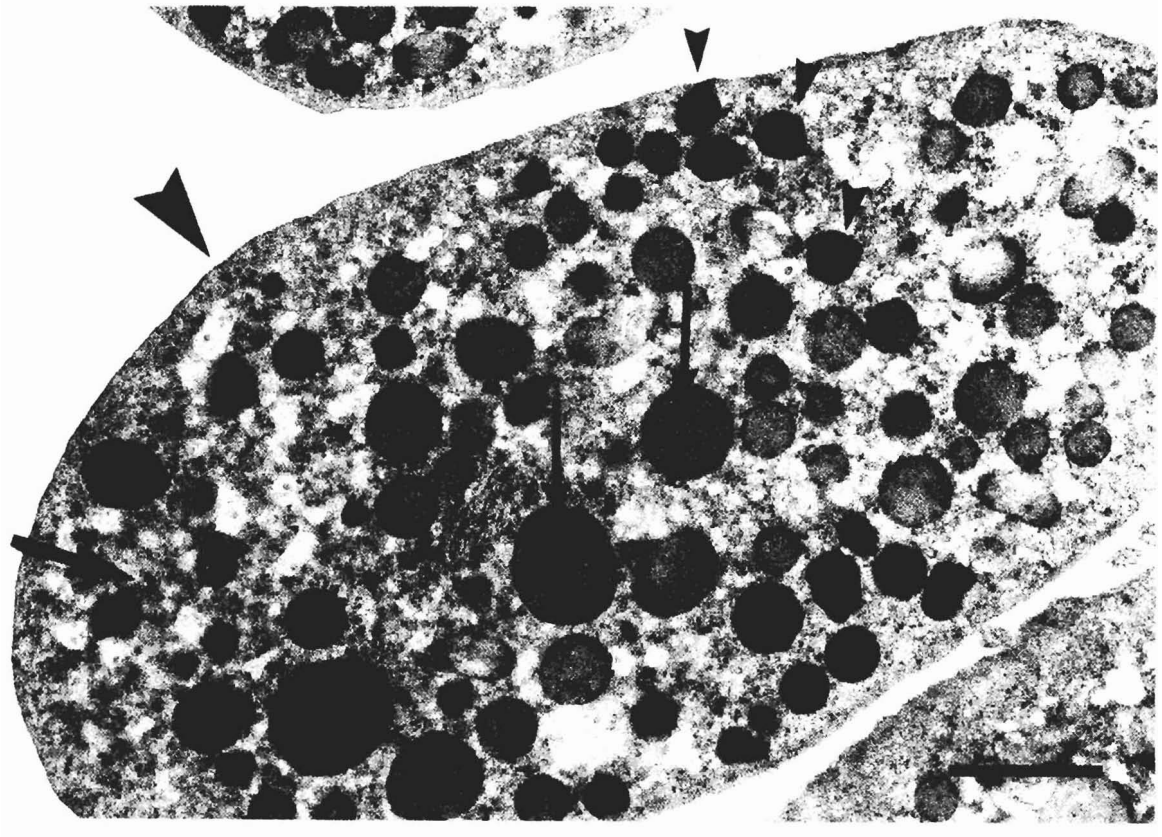

Fig. 5. Clara cell (large arrowhead) showing two types of granules (osmiophilic, small arrowhead, and non-osmiophilic, small arrow) and smooth endoplasmic reticulum (large arrow), surrounded by microvesicles. Group I. Bar= 1 μm.

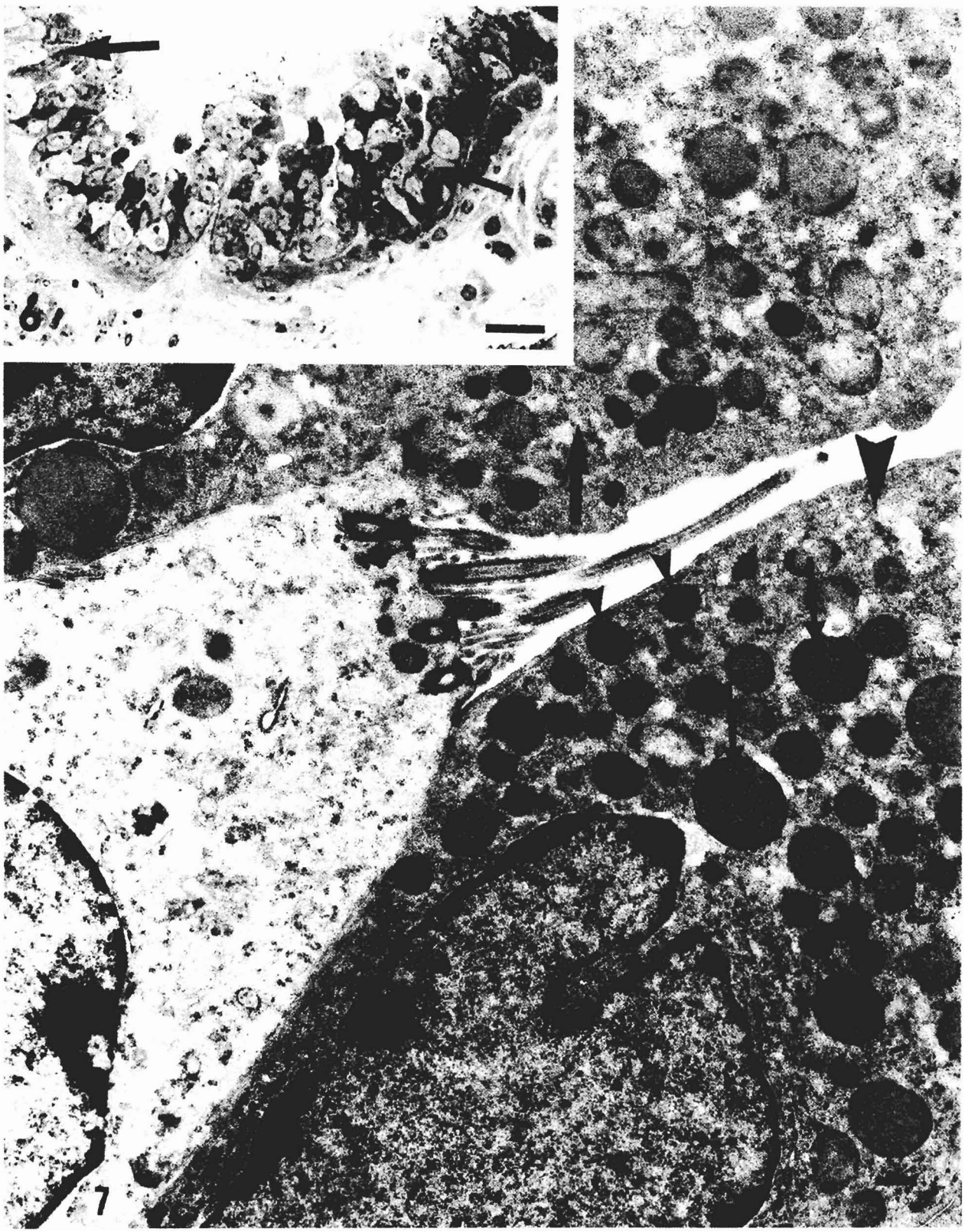

Fig. 6. Bronchiole with increased numbers of ciliated cells (small arrow) in comparison with Clara cells (large arrow). Group II. Bar= 30 μm.

Fig. 7. A bronchiole with Clara cells (large arrowhead) showing the two types of granulations (osmiophilic, small arrowhead, and non osmiophilic, small arrow) and normal smooth endoplasmic reticulum (large arrow). Group II. Bar= 1 μm.

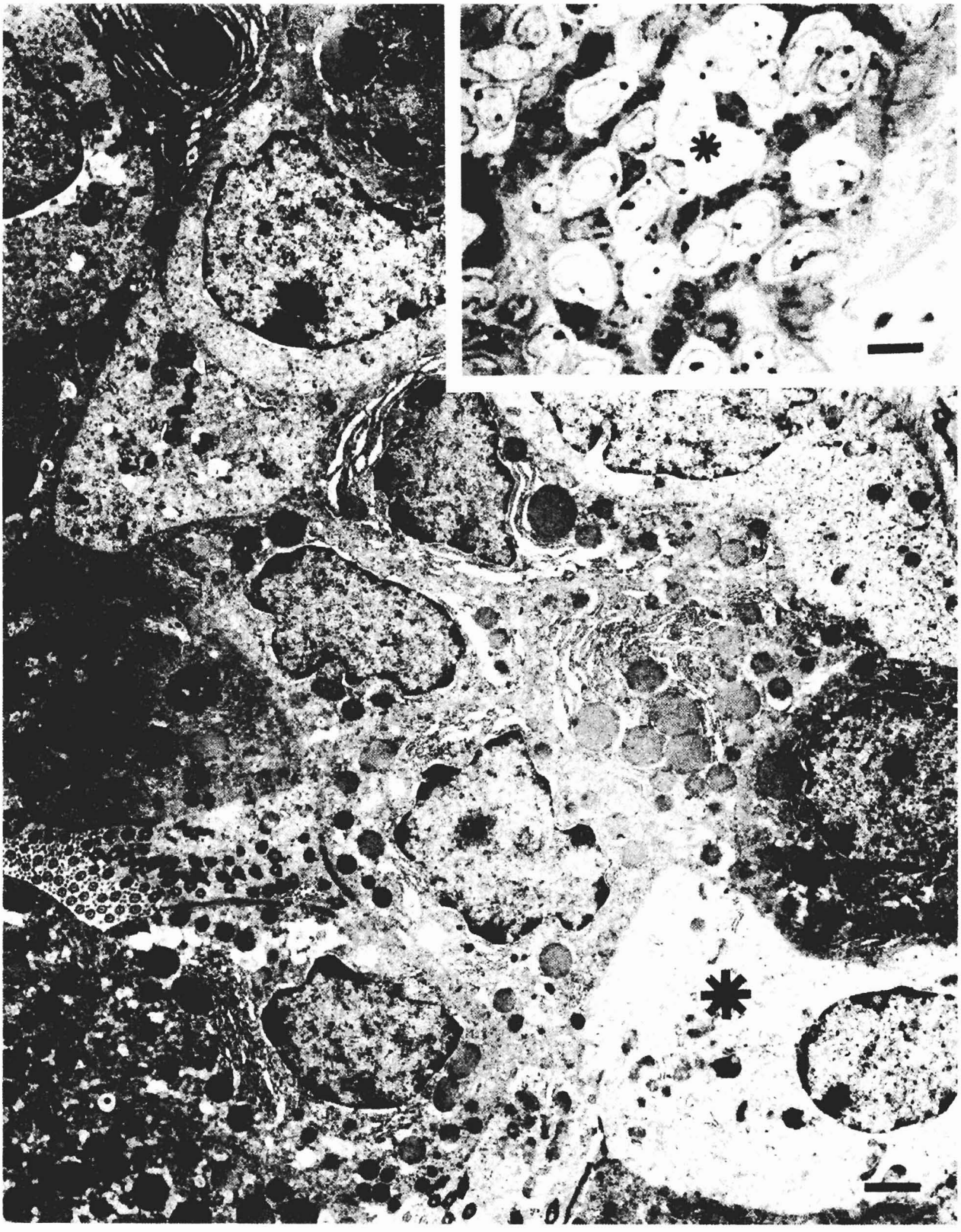

Fig. 8. In this bronchiole ciliated cells (asterisk) outnumber Clara cells (star). Group III. Bar = 10 μm.

Fig. 9. This figure shows abundant ciliated cells (asterisk) over Clara cells (star). Group III. Bar=1 μm.

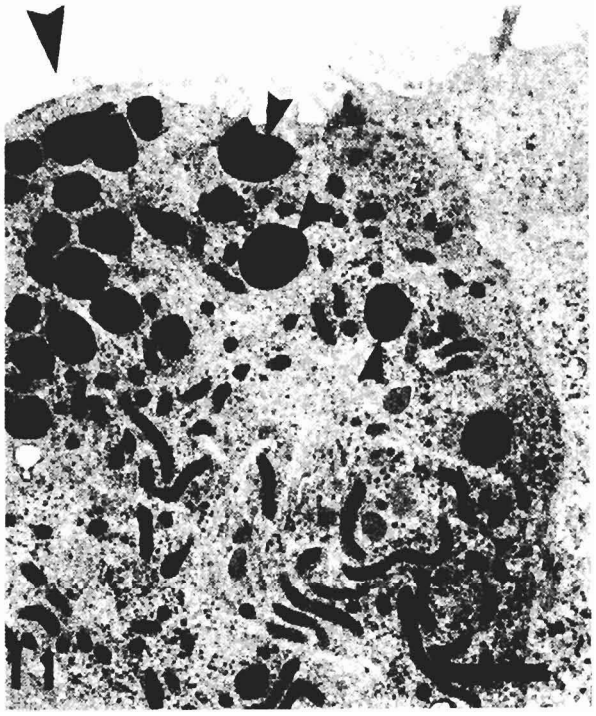

Fig. 10. This Clara cell (large arrowhead) shows vacuolation (v). Sometimes, they are related to numerous smooth endoplasmic reticulum microvesicles (small arrow). Group III. Bar= 2 μm.

Fig. 11. Detail of Clara cell (large arrowhead) of control group; we appreciate only one secretory granule (small arrowhead). Control group. Bar=0.5 μm.

different animal species; Plopper et al. (1980) in hamsters, guinea pigs and mice; and Kaup et al. (1985) in the horse; it is in contradiction with many other reports - and even with the results obtained for our own control group, viz. 15-day-old Swiss mice - in that the Clara cells contain, a single, small secretory granule of high electron density, which is consistent with the small granules observed in our work. On the other hand, our larger granule was previously unreported, so it can be regarded as a two-fold granular variation in Clara cells.

Of all the substances produced by Clara cells, only the isoenzymes of cytochrome P-450 reported by Devereux et al. (1985) are known for certain to be stored in the smooth endoplasmic reticulum. The storage sites for all other substances including the surfactants reported by Etherton et al. (1973) - and, particularly, Patton et al. (1991) - and the CC 10 proteins obtained by Hagen et al. (1990) remain unknown. On this basis, the composition of the typical secretory granule of Clara cells remains uncertain, so we may not really be able to advance the composition of the two types of granules that we encountered in the above reports. In any case, our dense granules are similar to the secretory granule of Clara cells found in various animal species. On the other hand, our light electron dense granules may store some of the above-mentioned substances or, hypothetically, an unknown substance that might play a specific role in foetal bronchioles.

Both our results for 15-day-old Swiss mice and previous reports on various animal species under seemingly normal conditions (e.g. Massaro et al. 1981) revealed the occurrence of scant Clara cells in the bronchiolar wall. This contradicts our results for mouse foetuses as their bronchioles were found to contain Clara cells in higher proportions than ciliated cells. Our results are very similar to those found by Strum et al., (1990) in hamster foetuses, which showed increased numbers of Clara cells that diminished immediately on birth. Such an increase and subsequent decrease in the number of Clara cells may be the result of such cells and the substances they produce playing an active role in foetal bronchiolar metabolism. As stated by Devereux et al. (1985), these cells take part in the biotransformation of xenobiotic substances via their cytochrome P-450 isoenzymes; hence, their numbers increase significantly in toxic processes [e.g. in toxic bronchitis in the horse, as reported by Drommer et al. (1987)]. Our bronchioles may thus have contained some unknown blood-borne xenobiotic substances, as previously indicated by Kanekal et al. (1990), which might be responsible for the increased numbers of Clara cells found.

The decrease in the number of Clara cells detected throughout the experiments was accompanied by degradation images that fostered cells scaling. As pointed out by Kanekal et al. (1990), this may be the result of the degenerative and necrotizing activity of free radicals arising from the biotransformation of xenobiotic substances.

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