ULTRASTRUCTURE OF THE NONCILIATED BRONCHIOLAR EPITHELIAL (CLARA) CELLS IN THE LUNGS OF NEONATAL RABBITS

Marei, H. E. S. * and El-Habback, H. A. **

*Departments of Cytology and Histology, Mansoura**

*and Cairo**Universities, Egypt*

ABSTRACT

Clara cells are non-ciliated one of airway stem cells that have been demonstrated in the bronchioles of most examined adult mammalian species. Certain features concerning the ultrastructural morphology and the way by which their secretory granules are released from the cytoplasm are still a matter of much dispute. The purpose of this study was to assess in conducting airways of normal neonatal rabbit lungs: (1) the cellular morphology and ultrastructural peculiarities of Clara cells; and (2) the way by which their secretory granules are released. Samples of histologic normal tissue were taken from ten lungs obtained from one-day-old rabbits and were processed according to standard techniques of light and transmission electron microscopy. The Clara cells had well-developed apical protrusions packed with vesiculated smooth endoplasmic reticulum and numerous electron-dense granules. Ciliary body-like structures were observed in some Clara cells. The granules of Clara cells were spherical or oval and their matrix was composed of both dense and of less electron-dense materials. Some of them were encountered to be located immediately below the luminal membrane. Morphological evidence for both merocrine and apocrine mode of secretion was demonstrated. It is suggested that the neonatal rabbit Clara cells may play crucial roles both as secretory and progenitor cells in the normal epithelium of the distal conducting airways in neonatal rabbits.

INTRODUCTION

The Clara cells are non-ciliated and non-mucous epithelial cells within the lining of distal conducting airways. They were first described in the human respiratory epithelium of the peripheral conducting airways **(Clara, 1937)**, and were defined mainly by their distinctive cytoplasmic granules, indicating a secretory function. Numerous electron microscopic studies performed in

mammals have shown considerable interspecies heterogeneity in both morphology and distribution of these cells **(Plopper et al., 1990; Plopper et al., 1991)**. An ultrastructural characteristic of Clara cells is the presence of electron-dense membrane-bound granules **(Bedetti et al., 1987)**. The Clara cell secretory granules have been shown to vary considerably in size, shapes and texture of the contents **(Kuhn et al., 1979)**. Several biochemical and immunohistochemical studies **(Singh and Katyal, 1984; Singh et al., 1985 a,b; Bedetii et al., 1987)** have identified three rat Clara cell-specific proteins.

The Clara cells have been found to contribute to cell renewal in hamster bronchial epithelium in the steady state **(Breuer et al., 1990)**. The proliferative response of the bronchiolar epithelium after exposure of rats to NO2 **(Evans et al., 1978)** or O3 **(Lum et al., 1978)**) gases is predominantly due to Clara cell division. Grafting isolate cell fractions of rabbit bronchiolar epithelium highly enriched in Clara cells onto denuded tracheas results in an epithelium containing both Clara cells and ciliated cells, resembling bronchiolar epithelium **(Nettesheim et al., 1990)**. Thus, the Clara cell is an important cell type regarding cell renewal in conducting airway epithelium in both health and disease **(Shami, 1989)**.

To our knowledge, few ultrastructural studies have been devoted to elucidates the ultrastructural morphology of neonatal rabbit Clara Cells. Thus, the aim of the present study was to study in normal neonatal rabbit airway epithelium, the ultrastructural peculiarities of Clara cells. Such data might be crucial for species comparison, for elucidating developmental events, and for confirming their suggested functional role such as their contribution to the proliferation compartment and their role as progenitor cells within the distal conducting airways.

MATERIAL AND METHODS

Ten one-day-old apparently healthy New Zealand White rabbits were collected from a local farm. Anaesthesia was induced with sodium pentobarbitone (60 mg/kg, IP). Their chests were opened and samples were taken from the different regions of the cranial and caudal lung lobes. For light microscopy, tissues were fixed in 10% neutral buffered formalin or Bouin's fluid. They were then dehydrated and embedded in paraffin. Paraffin sections of 5-6µm thick were prepared and stained by Harris Hematoxylin and Eosin (H&E) for general studies, the periodic acid/Schiff (PAS) technique (with and without saliva digestion) to demonstrate glycogen and neutral mucosubstances and Best's carmine for confirming the presence of glycogen. For electron microscopy, selected lung pieces were fixed for 12 hours in 2.5% glutaraldehyde in cacodylate buffer adjusted to pH 7.4. After rinsing with the same buffer, the tissues were postfixed in 1% buffered osmium tetroxide for 2.5 h at 4°C. The specimens were then quickly rinsed in buffer and dehydrated in a

graded series of ethanol, and were embedded in poly bed 812. Semithin sections were cut and stained with an aqueous solution of 1% toluidine blue for light microscopic orientation and selection of terminal bronchioles. Thin sections of the terminal bronchioles were mounted on Formvar-coated copper grids and double stained with uranyl acetate and lead citrate and examined with a JEOL 100c transmission EM at 80 kv.

RESULTS

By light microscopy, the Clara cell was cuboidal to columnar in appearance with a spherical nucleus and a dome-shaped apical projection (Fig. 1). The cytoplasm of the Clara cells generally displayed a negative PAS (Fig. 2) and Best's carmine reactivity.

By electron microscopy, The Clara cells had a cytoplasm of moderate electron density. The nucleus was basal, elongated and had a variable complexity of form. The nuclear contour was infolded (Fig. 3). The apical portion of the cell invariable projected into the lumen of the airways and was packed with numerous vesiculated smooth endoplasmic reticulum (Fig. 4). The granules of Clara cells were readily identified as electron-dense structures located in the apical cytoplasm (Figs. 4, 5, 6).

The granules of the Clara cells were spherical or oval in shape and membrane-bounded (Fig. 4, 5, 6). Their matrix was composed both of dense and of less electron-dense materials. The distributional pattern of these two materials was variable from one granule to another. The limiting membrane of the granules showed a regular contour (Fig. 4, 5, 6). Some granules were encountered to be located immediately below the luminal membrane (Fig. 6). The close proximity of some of these granules to the apical membrane might suggest extrusion of these electron-dense vesicles by a merocrine secretory process. In some section, apocrine secretion was indicated by extension of the apical cytoplasmic projection of the Clara cell into the airway lumen (Fig. 6). The luminal projection of the cell usually bore numerous short microvilli (Fig. 6). A well developed junction complex joined the Clara cells to their neighboring cells (Fig. 4).

In some Clara cells, the cytoplasm contained many free ribosomes. The luminal surface of the cell invariably bore microvilli, although the apical projection was not always pronounced (Fig.7). In these cells, smooth endoplasmic reticulum was limited. Large areas of rough endoplasmic reticulum were seen, usually in the apical portion of the cytoplasm (Fig. 7). Few electron-dense secretory vesicles were encountered (Fig. 7). These cells were connected to adjacent cell by a well-developed junctional complex (Fig. 7).

In some other sections, the Clara cell besides having the characteristic aforementioned ultrastructural morphology, they contained basal bodies-like structures that were encountered pri-

marily within the apical cytoplasm (Fig. 8, 9). In these cells the smooth endoplasmic reticulum seemed to be breaking down to form vesiculated areas (Fig. 8, 9).

DISCUSSION

In the present study, the cytoplasm of the neonatal rabbit Clara cells generally displayed a negative PAS and Best's carmine reactivity. There is much disagreement in the literature as to the reaction of Clara cells to mucus stains, particularly PAS. It is generally accepted that Clara cell do not stain with PAS **(Niden, 1967; Kuhn et al., 1979)**. In contrast to the present results, some authors have demonstrated granules in Clara cells with PAS **(Azzopardi and Thurlbeck, 1969; Roth, 1973)** or its electron microscopic equivalent PATO **(Cutz and Conen, 1971)**. Based on PAS and Best's carmine reactivity as well as the ultrastructure criteria, the present study provided histochemical and ultrastructural confirmation for the lack of glycogen in the Clara cells of the neonatal rabbit. In contrast to our findings, **Massaro and Massaro (1986)** provided a histochemical evidence of the presence of glycogen in Clara cells of 1-day-old rats. Glycogen has been reported also in the dog **(Plopper et al., 1980)**, neonatal pigs **(Baskerville, 1970)**, in 3- and 12-month-old calves **(Smith et al., 1979)**, and in neonatal 2-day-old calf **(El-Gawad and Westfall, 1997)**. The abundant amount of glycogen is considered a sign of immaturity in Clara cells of the rat **(Massaro and Massaro, 1986; Ji et al., 1995)**, mouse **(Ten-Have-Opbrock and De Vries, 1993)**, rabbit **(Plopper et al., 1983)**, and human **(Jeffery et al., 1992)**. The lack of glycogen in the Clara cells of the neonatal rabbit that has been revealed in the present study might suggest that the neonatal rabbit Clara cells are probably more differentiated and more matured in comparison to other animal species.

Based on their ultrastructural features (the presence of apical protrusions, basal bodies, secretory granules and sER), various morphologically distinct Clara cell populations were recognized in the present study. In some Clara cells, a well pronounced apical protrusion filled with a significant number of sER and a considerable number of electron-dense granules were observed. Other cells have no apical protrusions and their apical cytoplasm contains either no or very few sER tubules, very few numbers of electron-dense granules and large areas of rough endoplasmic reticulum. Still other Clara cells appeared to have features intermediate between the two aforementioned extremes and are mainly recognized through the presence of basal body-like structures within their apical cytoplasm. Our subclassification of Clara cell, though arbitrary, is based on definitive ultrastructural characteristics. Although many of our identifying criteria are consistent with those of other investigators such as **Christensen et al. (1987)** who demonstrated the presence of at least three types of Clara cells in the hamster, there is no standardization in the literature for the classification of Clara cells. In the neonatal calf, only one type of Clara

cell, with a centrally located indented nucleus has lateral and apical strands of rER and a prominent apical aggregation of large, pale granules was identified **(El-Gawad and Westfall, 1997)**.

The question of whether these morphologically distinct forms of Clara cells are considered as specific cell types or as different functional phases of the same cell type has not been elucidated yet. In our opinion, the three morphologically distinct Clara cell types in neonatal rabbit that have been demonstrated in the present study could be interpreted as different developmental phases for one cell type. The rER tubules seem to be involved in the formation of electron-dense granules that accumulate in the apical cytoplasm. These findings were in accord with those of **Kuhn et al. (1979)** who clarified that the differentiation of protein synthesizing rER suggested an earlier stage of development leading to formation of secretory granules by the Golgi complex in Clara cells of the rat. The ultrastructural peculiarities of the Clara cells presented here fit well with those reported for the Clara cells of many other species. In this respect, numerous ovoid secretory granules and an abundance of agranular ER (sER) have been reported in most Clara cells **(Reid and Jones, 1979; Plopper et al., 1983; Breeze and Turk, 1984; Christensen et al, 1987)**.

There is a considerable variation in the ultrastructural features of Clara cells among different animal species. In contrast to the present results that have demonstrated the existence of a considerable amount of sER in the apical region of neonatal rabbit Clara cells, **El-Gawad and Westfall (1997)** have observed a minimal amount of sER in Clara cells of neonatal calves. Sparse amounts of sER also were demonstrated in Clara cells of adult cattle, dog **(Plopper et al., 1980)** and human **(Jeffery et al., 1992)**.

The cellular morphology of the neonatal rabbit Clara cells (abundant sER and numerous electron dense granules) revealed in the present study might suggest active detoxification and secretory roles of these cells. The lack of significant amounts of sER in Clara cells of neonatal calves suggested that these cells do not play an important role in detoxification in this species **(El-Gawad and Westfall, 1997)**. It has been suggested that the Clara cells are secretory cells and produce surfactant **(Niden, 1967)**, or that they produce hypophase **(Petrik and Collet, 1974)**.

The ways by which the electron dense granules are released from the cells have not been determined yet. Most of the electron-dense granules of the neonatal rabbit Clara cells were localized either in a supranuclear position or lying immediately below the luminal cell membrane. In some cells, apocrine secretion was also indicated by extension of the apical cytoplasmic projection of the Clara cell into the airway lumen. Such findings might indicate the release of these granules both by merocrine and apocrine mode of secretion. Some morphological studies have indicated that these electron-dense granules may be lost from the cell by merocrine secretion

(Stinson and Loosli, 1978; Al-Ugaily et al., 1980; Pack et al., 1981; Yoneda and Birk, 1981). El-Gawad and Westfall (1997) suggested that the presence of apical granules in Clara cells of the neonatal calf suggest a secretory function, which is presumably merocrine at this early stage of development. Several studies **(Heath et al., 1976; Etherton et al., 1979; Pack et al., 1980)** have suggested that Clara cell may also undergo apocrine secretion. It has been proposed that this apical cytoplasmic bleb is a fixation artifact **(Jeffery and Reid 1977)**. Other studies using several preservation techniques indicate that this is not the case, and that these profiles may genuinely represent apocrine secretion **(Etherton et al., 1979)**. Some authors suggest that both apocrine and merocrine secretions occur in Clara cells **(Stinson and Loosli, 1978; Pack et al., 1981; Peao et al., 1993)**.

The granules of the Clara cells of the neonatal rabbit that were demonstrated in the present study were similar to those described by **Stinson and Loosli (1978)** in the Clara cells of the terminal bronchioles of mice. Many authors **(Etherton et al., 1979; Evans et al., 1975)** consider the Clara cell granules to be mitochondria and it has been suggested on the basis of autoradiographic evidence **(Etherton et al., 1979)** that these "mitochondria of an unusual structure" produce long chain fatty acids.

Our observations indicate that Clara cells may develop cilia whilst in the process of secretion. It is, therefore, conceivable that both Clara and ciliated cells are progressive stages in epithelial turnover. In addition to a secretory role, it has also been proposed that the Clara cell is a precursor of the other types of epithelial cell **(Evans et al., 1978)**. Cells with Clara cell-like cytoplasmic inclusions bearing cilia were seen **(Pack et al., 1980)**. Thus Clara cells may undergo metamorphosis to become ciliated cells. A clear progenitor relationship between the bronchiolar Clara cells and ciliated cells has previously been demonstrated in the terminal bronchioles of neonatal calves **(Marei and El-Gawad, 2001)**.

Fig 1 : Light photomicrograph of neonatal rabbit bronchiolar epithelium showing Clara cell (C) (H&E X 1000).

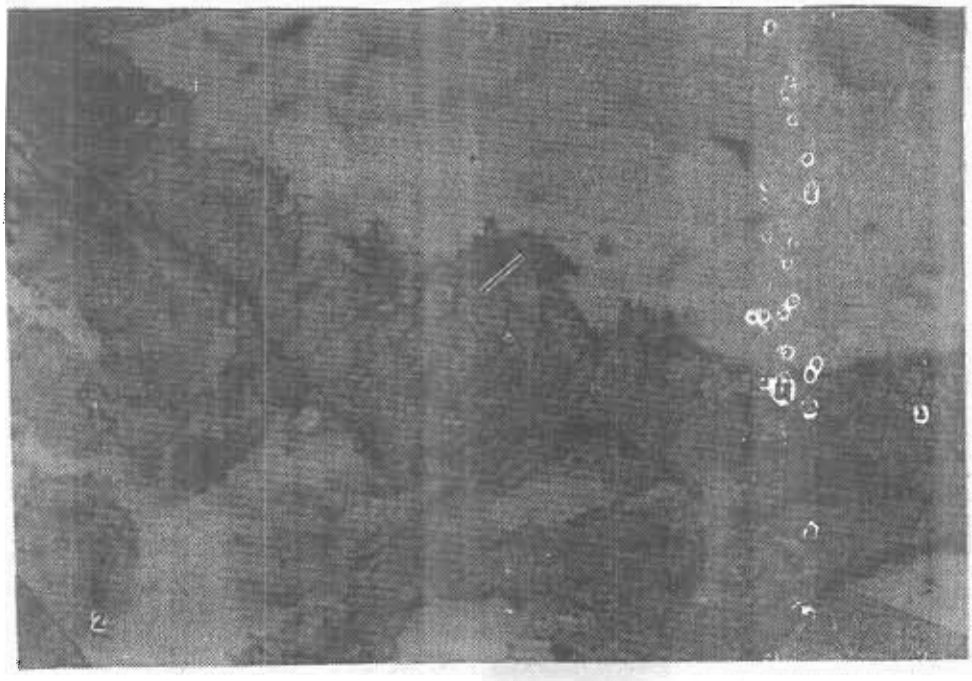

Fig 2 : Light photomicrograph of neonatal rabbit bronchiolar epithelium showing negative PAS reaction in the cytoplasm of Clara cell (arrow) (PASX 1000)

Fig 3 : General view for neonatal abbit Clara cell (C). Note nucleus (N) and apical protrusion (A) (X 3600).

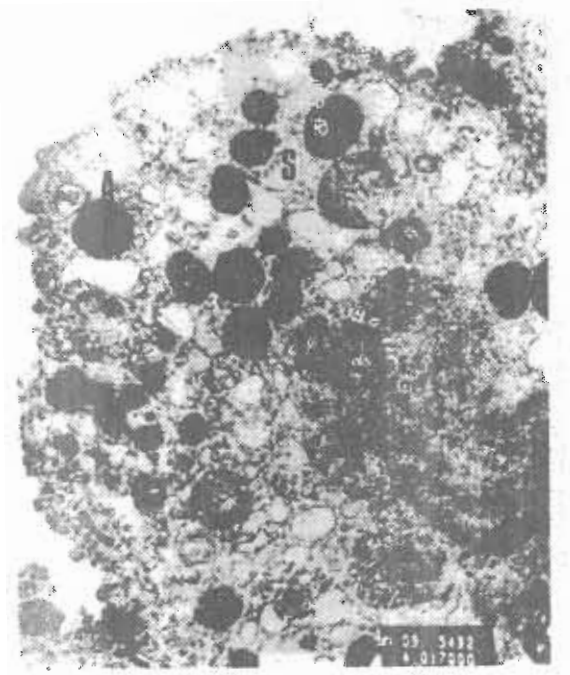

Fig 4 : Neonatal rabbit Clara cell. Note nucleus (N), Clara cell granules (A), sER (S), and junctional complex (j). (X 17000).

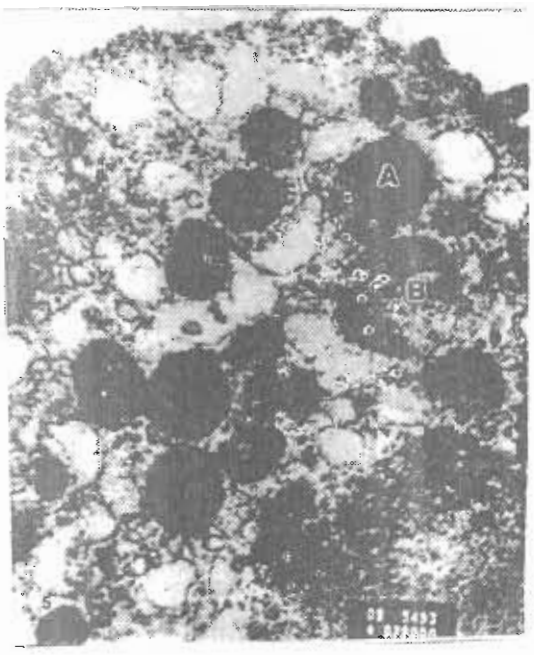

Fig 5 : Neonatal rabbit Clara cell at a higher magnification. Note Clara cell granule (A). (X 28000).

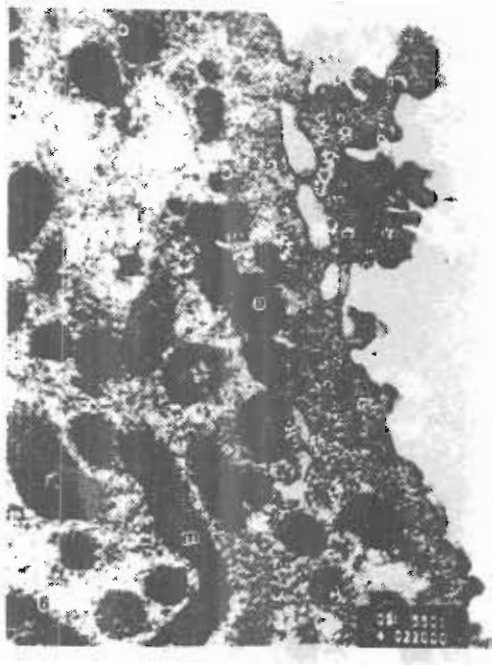

Fig. 6 : Neonatal rabbit Clara cell. Note the close proximity between Clara cell granules (a) and the luminal plasma membrane. Note also mitochondria (m) and bleb-like apical protrusion (arrow) (X 22000).

Fig. 7 : Neonatal rabbit Clara cell (C). Note nucleus (N), only one dense granules (G), rER (E) and junctional comples (J) (X 22000).

Fig. 8 : General view for neonatal rabbit Clara cell (C). Note nucleus (N), and basal bodie-like structures (b) in the apiecal cytoplasm (X 13000).

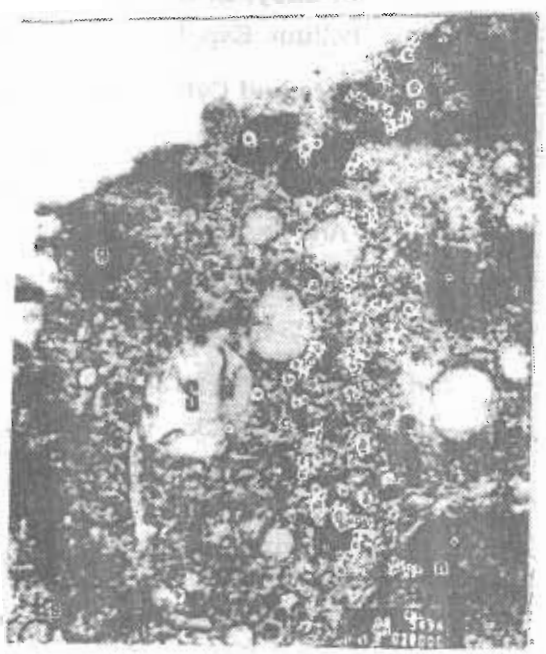

Fig. 9 : A higer magnificantion for neonatal rabbit Clara cell (C). Note nucleus (N), sER (S), dense granules (G) and basal bodie-like structures (b) (X 28000).

REFERENCES

Al-Ugaily, L. H.; Morris, G.; Pack, R. J. and Widdicome, J. G. (1980) : Clara cells in the airway epithelium of the mouse. J. Phys. 303:43-44.

Azzopardi, A. and Thurlbeck, W. M. (1969) : The histochemistry of the nonciliated bronchiolar epithelial cells. Am. Rev. of Res. Dis. 99, 516-525.

Baskerville, A. (1970) : Ultrastructure studies of the normal pulmonary tissue of the pig. Res. Vet. Sci. 11: 150-155.

Bedetti, C. D., Singh, J.; Singh, G.; Katyal, S. L. and Wong-Chong, M. L. (1987) : Ultrastructural localization of rat Clara cell 10 kd secretory protein by the immunogold technique using polyclonal and monoclonal antibodies. J. Histochem. Cytochem. 35: 789-794.

Breeze, R. and Turk, M. (1984) : Cellular structure, function and organization in the lower respiratory tract. Environ. Health Perspect, 55:3-24.

Breuer, R; Zajicek, G.; Christensen, T. G.; Lucey, E. C. and Snider, G. L. (1990) : Cell kinetics of normal adult hamster bronchial epithelium. Am. J. Respir. Cell Mol. Biol. 2: 51-58.

Clara, M. (1937) : Zur Histobiologie des Bronchialepithels. Z. mikrosk-anat Forsch 4: 321-347.

Christensen, T. G.; Breuer, L.; Hornstra, L. J.; Lucy, E. C. and Snider, G. L. (1987) : The ultrastructure of hamster bronchial epithelium. Exp. Lung Res. 13:253-277.

Cutz, E. and Conen, P. E. (1971) : Ultrastructure and Cytochemistry of Clara cells. Am. J. Pathology 62:127-141.

El-Gawad, M. A. and Westfall, J. A. (1997) : Electron microscopy of nonciliated bronchiolar (Clara) cells in neonatak calves. Proce. Am. Assoc. Vet. Anat.

Etherton, J. E.; Purchase, I. F. and Corrie, B. (1979) : Apocrine secretion in the terminal bronchiole of mouse lung. J. Anat. 129: 305-322.

Evans, M. J.; Cabral-Anderson, L. G. and Freeman, G. (1978) : Role of the Clara cell in the renewal of the bronchiolar epithelium. Lab Invest. 38: 648-653.

Evans, M. J.; Greenburg, S. D.; Lewis, C. W. and Levy, M. L. (1975) : The Clara cell: A brief review with illustrative ultrastructure. Patologia. 13:463-469.

Heath, D.; Smith, P. and Harris, P. (1976) : Clara cells in the Lama. Expth. Cell Biol. 44:73-82.

Jeffery, P. K.; Gaillard, D. and Moret, S. (1992) : Human airway secretory cells during development and in mature airway epithelium. Eur. Respir. J. 5:93-104.

Jeffery, P. K. and Reid, L. M. (1977) : The respiratory mucous membrane. In Respiratory de-

fense mechanisms (ed. JD Brain, DF Proctor & LM Reid). New York:Marcel Dekker

Ji, C. M.; Plopper, C. G. and Pinkerton, K. E. (1995) : Clara cell heterogeneity in differentiation: correlation with proliferation, ultrastructure composition and cell position in the rat bronchiole. Am .J. Respir. Cell Mol. Biol.3: 144-151

Kuhn, C.; Callaway, L. A. and Askin, F. B. (1979) : The formation of granules in the bronchiolar Clara cells of the rat. I. Electron microscopy J. Ultrastruc. Res. 49:387-400

Lum, H.; Schartz, L. W.; Dungworth, D. L. and Tyler, W. S. (1978) : A comparative study of cell renewal after exposure to ozone or oxygen. Am. Rev. Respir. Dis. 118: 335-345

Marei, H. S. and El-Gawad, M. A. (2002) : Differentiation of ciliated cells in the terminal bronchioles of neonatal calves. Euro. J. Morph. 39/5:269-276

Massaro, G. D. and Massaro, D. (1986) : Development of bronchiolar epithelium in rats. Am. J. Physiol. 250:R783-R788

Nettesheim, P.; Jetten, A. M.; Inayama, Y.; Brody, A. R.; Gray, A. T.; Mahler, J. F. and Hook, G. E. (1990) : The role of Clara cells and basal cells as epithelial stem cells of the conducting airways. In D. G. Thomassen and P. Nettesheim, editors. Biology, Toxicology and Carcinogenesis in the Respiratory Epithelium. Hemisphere, New York, 99-111.

Niden, A. H. (1967) : Bronchiolar and large alveolar cell in pulmonary phospholipid metabolism. Science 158, 1322-1324.

Pack, R. J.; Al-Ugaily, L. H. and Morris, G. (1981) : The cells of the tracheobronchial epithelium of the mouse: a quantitative light and electron microscopy study. J. Anat. 132:71-84.

Pack, R. J.; Al-Ugaily, L. H.; Morris, G. and Widdicombe, J. G. (1980) : The distribution and structure of cells in the tracheal epithelium of the mouse. Cell and Tissue Research 208: 65-84.

Peao, M. N.; Aguas, A. P.; DeSa, C. M. and Grande, N. R. (1993) : Anatomy of Clara cell secretion: surface changes observed by scanning electron microscopy. J. Anat. 183:377-388

Pertrik, P. and Collet, A. J. (1974) : Quantitative electron microscopic autoradiography of in vitro incorporation of 3 H-choline, 3 Hleucine, 3 H-acetate and 3 H-galactose in nonciliated bronchiolar (Clara) cell of mice. Am. J. Anat. 139:519-534.

Plopper, G. C.; Aleey, C. J.; Serabjit-Singh, C. J. and Philpot, R. M. (1983) : Cytodifferentiation of the nonciliated bronchiolar epithelial (Clara) cell during rabbit lung maturation: An ultrastructural and morphometric study. Am. J. Anat. 167:329-357.

Plopper, C. G.; George, S. T.; Pinkerton, K. E.; Tyler, N.; Mariassy, A.; Wilson, D.; Wu, R.; Hyde, D. M. and Evans, M. J. (1990) : Tracheobronchial epithelium in vivo: composition, differentiation and response to hormones. In D. G. Thomassen and P. Nettesheim, editors. Biology, Toxicology and Carcinogenesis in the Respiratory Epithelium. Hemisphere, New York. 308

Plopper, C. G.; Hyde, D. M.; Buckpitt, A. R. (1991) : Clara cells. In R. G. Crystal and J. B. West, editors. The Lung: Scientific Foundations. Raven Press, New York. 215-228.

Plopper, G.G.; Mariassy, A.T. and Hill, L. H. (1980) : Ultrastructure of the nonciliated bronchiolar epithelia (Clara) cell of mammalian lung. I. A comparison of rabbit, guinea pig, rat, hamster, and mouse. Exp. Lung Res. 1:139-154

Reid, L. and Jones, R. (1979) : Bronchial mucosal cells. Fed Proc 38.191-196

Roth, J. (1973) : The Clara cells and the pulmonary surfactant system. Experimentelle Pathologie 2:305-313

Shami, S. G. (1989) : Lung cell kinetics. In D. Massaro, editor. Lung Cell Biology. Marcel Dekker, New York. 1-37

Singh, G. and Katyal, S.L. (1984) : An immunologic study of the secretory products of rat Clara cells. J. Histochem. 32:49-54

Singh, G.; Katyal, S. L. and Gottron, S. A. (1985a) : Antigenic, molecular and functional heterogeneity of Clara cell secretory proteins in the rat. Biochim. Acta 829:156-163

Singh, G.; Katyal, S. L.; Ward, J. M.; Gottron, S. A.; Wong-Chong, M.; Riley, E. J. (1985b) : Secretory proteins of the lung in rodents:immunocytochemistry. J. Histochem. Cytochem. 33:564-568

Smith, M. N.; Greenber, S. D. and Spjut, H. J. (1979) : The Clara cell: A comparative ultrastructural study in mammals. Am. J. Anat. 155:15-30

Stinson, S.F. and Loosli, C. G. (1978) : Ultrastructural evidence concerning the mode of secretion of electron-dense granules by Clara cells. J. Anat. 127:291-298

Ten Have-Opbrock, A. W. and De Vries, E. P. (1993) : Clara cell differentiation in the mouse: ultrastructural morphology and cytochemistry of surfactant protein A and Clara cell 10 k D protein. Microsc. Res. And Tech. 26: 400-411.

Yoneda, K. and Birk, M. G. (1981) : The mode of secetion of the Clara cell in rat bronchiole: a freeze fracture study. Exp. Lung Res. 2: 77-185.

الملخص العربى

دراسة التركيب الدقيق للخلايا غير الهدبية (خلايا كلارا) فى النسيج الطلائى للشعيبات الهوائية فى رئة الأرانب حديثة الولادة

هانى السيد مرعى* هانى أحمد الحباك**

كلية الطب البيطرى جامعة المنصورة* وجامعة القاهرة**

تعتبر خلايا الكلارا من الخلايا غير الهدبية المتواجدة فى النسيج الطلائى المبطن للشعيبات الهوائية فى الثدييات والحيوانات المختلفة. الغرض من هذه الدراسة هو التعرف على التركيب الدقيق ومحاولة الوصول إلى الوظائف المحتملة لتلك الخلايا من خلال دراسة الخواص الشكلية لتلك الخلايا. أجريت هذه الدراسة على عينات أخذت من رئات عدد عشرة أرانب حديثة الولادة عمر يوم واحد حيث تم تجهيز تلك العينات للدراسة والفحص بواسطة الميكروسكوب الضوئى والإلكترونى. كما تم دراسة بعض الخصائص الهستوكيميائية لتلك الخلايا على مستوى الميكروسكوب الضوئى بعد صبغها بكل من صبغة الـ PAS والـ Best s carmine أظهرت تلك الدراسة تفاعلاً سلبياً لسيتوبلازم خلايا الكلارا مع كل من صبغتى PAS و Best s carmine مما يشير إلى خلو سيتوبلازم تلك الخلايا من الجليكوجين، وعلى مستوى الميكروسكوب الإلكترونى تميزت خلايا الكلارا بوجود بروزات قمية التى امتلئت بكمية كبيرة من أنابيب الشبكة الإندوبلازمية الناعمة والعديد من الأجسام القاعدية المتشابهة بالأهداب أسفل أسطح بعض الخلايا. فى هذه الدراسة تم تمييز وجود العديد من الحويصلات الإفرازية فى المناطق القريبة جداً من جدار بعض الخلايا كما تم تمييز بعض الدلائل الوصفية التى من الممكن أن ترجح الطريقة التى يتم بها إفراز تلك الحويصلات إلى خارج سيتوبلازم تلك الخلايا، ومن خلال تلك الدراسة تم ترجيح الدور الوظيفى لتلك الخلايا حيث أوضحت هذه الدراسة قيام تلك الخلايا بالعديد من الوظائف الحيوية كخلايا إفرازية وكذلك كخلايا مولدة ومجددة للنسيج الطلائى المبطن للشعيبات الهوائية فى الرئتين.