TRANSFORMATION OF ALVEOLAR EPITHELIUM DURING EMBRYOGENESIS AND IN VARIOUS AGE PERIODS OF POSTFETAL LIFE IN RATS

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Cells of the alveolar epithelium during the period of formation of the respiratory portions contain numerous glycogen inclusions and possess high alkaline-phosphatase activity. In adult rats the cells of the alveolar epithelium with tiny glycogen granules in their cytoplasm and a higher alkaline phosphatase activity must be regarded as cambial in character. In the course of differentiation of cells of the alveolar epithelium, the content of RNA and of certain enzymes in their cytoplasm is increased. Some of these cells are converted into dust cells.

Most workers consider that the cell lining of the alveoli is epithelial in nature [1,2,6,10]. Electron-microscopic investigations confirm this view [11,13]. The character of the epithelial lining of the alveoli (continuous or interrupted), the presence or absence of alveolar pores [3,8], and also the nature of the alveolar cells found both in interalveolar septa and inside the cavity of the alveoli and possessing phagocytic activity remain subjects for debate. Some workers regard them as derivatives of the alveolar epithelium [1,9,12], while others consider that macrophages and dust cells are of connective-tissue origin [4,6].

In the present investigation, changes in the alveolar epithelium during the formation of the respiratory portions and at different age periods in adult rats were studied.

EXPERIMENTAL METHOD

The test object consisted of the lungs of rats during the period of embryogenesis and during postfetal life (newborn animals, rats aged 6, 12, 17, and 21 days, 1-12 months, and 1.5-2 years). Both the ordinary histological methods (fixation of the lungs in Zenker-formol, neutral formalin, and Carnoy's fluid, embedding in paraffin wax, and staining of the sections with hematoxylin-eosin, by Mallory's method, with silver by Gomori's method, with resorcin-fuchsin by Weigert's method) and histochemical methods (detection of labile oxidase by Graff's method, succinate dehydrogenase by the Seligman–Rutenberg method, alkaline and acid phosphatase by Gomori's method, glycogen by Shabadash's and Hotchkiss's method with diastase control, RNA by Unna's method, and lipids with Sudan black) were used. The number of cells in the interalveolar septa with a high content of these histochemically detected compounds also was counted.

EXPERIMENTAL RESULTS

Mass formation of primary alveolar sacs takes place at the end of embryogenesis on the 20th day. In corresponding areas of the lung parenchyma much of the mesenchyme is replaced by epithelial tissue. Primary alveolar sacs bud out from the intralobular epithelial tubes, representing the primitive bronchi, but initially they are not hollow. These sacs can be definitely detected on the 21st day of embryogenesis. By this time differentiation of the cells of the alveolar epithelium is apparent, as shown by the formation of single larger elements, with cytoplasm staining with the usual histological dyes, among the primary homogeneous cells with unstained cytoplasm. In the course of postfetal life, the number of larger cells in the interalveolar septa and in the lumen of the alveoli increases.

The cytoplasm in many cells is vacuolated. In addition, in cells lying inside the lumen of the alveoli, brown and black inclusions appear, i.e., they are transformed into dust cells, the number of which increases with age. Syncytia with two or sometimes more nuclei also are found in the lumen of the alveoli.

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The cells in the alveolar sacs undergo significant changes in their content of histochemically detectable compounds. During the formation of primary alveolar sacs all the cells composing them contain large masses of glycogen, and this is responsible for the fact that the cytoplasm of these cells does not stain with the usual histological dyes. As the alveolar sacs develop, the number of glycogen inclusions in their cells decreases, and in the alveolar sacs formed later, in subpleural areas, large masses of glycogen are concentrated. In the first days of postfetal life, many small granules of glycogen appear in the epithelial cells of the alveolar sacs, but during their subsequent subdivision, the number of cells with glycogen inclusions decreases, and after the 21st day only a few cells with small glycogen granules in their cytoplasm can be found in the interalveolar septa.

A uniform intensity of the reaction for alkaline phosphatase is observed in all cells in the epithelium of the primary alveolar sacs. In the course of subdivision of the alveolar sacs, a more intensive reaction for alkaline phosphatase persists in some groups of cells of the interalveolar septa. With age, the number of cells with higher alkaline phosphatase activity within the groups decreases, and in the interalveolar septa of old rats only solitary cells with higher activity of this enzyme remain.

In 22-day fetuses, single cells with RNA in their cytoplasm appear in the epithelium of the alveolar sacs: some are smaller in size, with cytoplasm staining intensively with pyronine, while the cytoplasm of other cells is larger but its degree of basophilia is smaller. In the course of postfetal life the number of cells with RNA in their cytoplasm in the interalveolar septa varies within narrow limits. With age, however, the number of smaller cells with a narrow rim of cytoplasm, possessing intense pyroninophilia, decreases and the number of larger cells with a smaller RNA content in their cytoplasm increases. The cytoplasm of these cells is partially vacuolated, because of the presence of lipids and acid phosphatase in it.

The intensity of the reaction for acid phosphatase is identical in all cells in the epithelium of the primary alveolar sacs of the embryos. In the first days after birth, single cells with clearly defined borders and giving an intense reaction for acid phosphatase can be seen in the interalveolar septa. Gradually and with fluctuations, their number increases to reach a maximum by the age of 7 months. In addition, the dimensions of the cells giving an intense reaction for acid phosphatase increase, and such cells are more frequently found in the lumen of the alveoli.

Labile oxidase in the form of occasional granules in the cytoplasm is uniformly distributed in all the cells of the primary alveolar sacs. When cavities appear in these sacs, cells with a much more marked reaction for labile oxidase are formed. The whole cytoplasm in these cells is filled with darkly stained granules, usually indistinguishable because of the great size of their contents. After birth, the number of these cells in the interalveolar septa increases to reach a maximum by 7 months.

Collections of cells with an intense reaction for labile oxygen are observed in areas of the lung parenchyma located around the pulmonary vein.

Succinate dehydrogenase is present in fetuses as tiny granules uniformly distributed in all the cells of the primary alveolar sacs. In the first months of postfetal life, the intensity of the reaction for succinate dehydrogenase in cells of the alveolar epithelium increases. In rats aged one month, among the majority of cells of the alveolar epithelium, single cells with clearly defined borders and with a high content of this enzyme can be distinguished. Fluctuations in the number of these cells at different age periods are relatively small. Succinate dehydrogenase activity in the great majority of cells of the alveolar epithelium decreases with age.

The following conclusions can be drawn from the results described. Since many glycogen inclusions are found in the epithelium of the alveolar sacs in the period of their formation, it may be considered that glycogen is not merely an energy-providing material, but also a plastic material [5]. The few cells with small glycogen granules and high alkaline phosphatase activity found in the interalveolar septa of adult rats must be regarded as cambial cells, responsible for physiological regeneration of the alveolar epithelium. In the course of differentiation of the cambial cell, RNA accumulates in their cytoplasm, causing an increase in size of these cells. The content of several enzymes also increases during differentiation. The appearance of cells with a more intense reaction for labile oxidase, acid phosphatase, and succinate dehydrogenase is preceded by a period in which the corresponding enzymes can be detected, although in smaller amounts, in the majority of cells of the alveolar epithelium, presumptive evidence of a genetic link between these cells. The increase in enzymic activity in the alveolar epithelial cells during their differentiation explains

their high reactivity, manifested in particular by the formation of dust cells and syncytia. The number of more highly differentiated cells in the alveolar epithelium increases with age.

LITERATURE CITED

1. N. G. Gorikov, Abstracts of Proceedings of a Scientific Conference on Determination and Plasticity of Tissues under Normal, Experimental, and Pathological Conditions, in memory of A. A. Zavarzin [in Russian], Leningrad (1959), p. 29.
2. G. Ya. Grafova, Arkh. Anat., Gistol. i Émbriol., No. 11, 67 (1965).
3. A. I. Klembovskii, Arkh. Anat., Gistol. i Émbriol., No. 3, 61 (1962).
4. S. T. Skoblenok, Development and Structure of the Epithelial Cover of the Pulmonary Alveoli in Man and Certain Mammals and on the Origin of Alveolar Macrophages, Author's abstract of doctorate dissertation, Novosibirsk (1951).
5. Yu. N. Shapovalov, Arkh. Anat., Gistol. i Émbriol., No. 1, 46 (1962).
6. S. I. Shchelkunov, Proceedings of the 5th All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], Leningrad (1951), p. 578.
7. H. A. Bickerman, in: A. Lansing (editor), Problems of Aging (1952).
8. S. Engel, Lung Structure, Springfield (1962).
9. T. Ito and S. Shibazaki, Arch. Histol. Jap., 25, 491 (1965).
10. A. Oppel, Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere, Vol. 6, Jena (1905).
11. H. Schulz, Die submikroskopische Anatomie und Pathologie der Lunge, Berlin (1959).
12. H. Westhues, Beitr. path. Anat., 70, 213 (1922).
13. H. Yasuda, Acta Path. Jap., 8, 189 (1958).