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EFFECT OF REMOVAL OF THE MATERNAL LUNG ON DEVELOPMENT OF THE FETAL LUNG IN RATS

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Pathology of an organ in a pregnant animal is known to disturb the development mainly of the homonymous organ of the fetus, although at the same time it leads to developmental disturbances of the other organs [1-6, 8]. There have been few experimental investigations of interaction between maternal and fetal lungs [7], and no information has been published concerning the structural changes taking place in the fetal lung in the case of a deficiency of lung tissue in the mother.

The aim of this investigation was accordingly to study the structural features of the lungs in fetuses obtained from unilaterally pneumonectomized rats.

EXPERIMENTAL METHOD

The lungs of Wistar rat fetuses were studied on the 18th and 21st days of intrauterine development. At each time in the experiment lungs were obtained from 10 fetuses — two each from the litter of a pneumonectomized mother. The operation was performed on the 9th day of pregnancy. Lungs of the same number of fetuses from five litters of intact rats were used as the control. In all the experiments tissue of the left fetal lung was studied histologically and electron-microscopically. The lungs were fixed in a 10% neutral formalin solution by Lillie's method and embedded in paraffin wax. Sections were stained with hematoxylin and eosin. Under the light microscope, photographs were taken of histological preparations under

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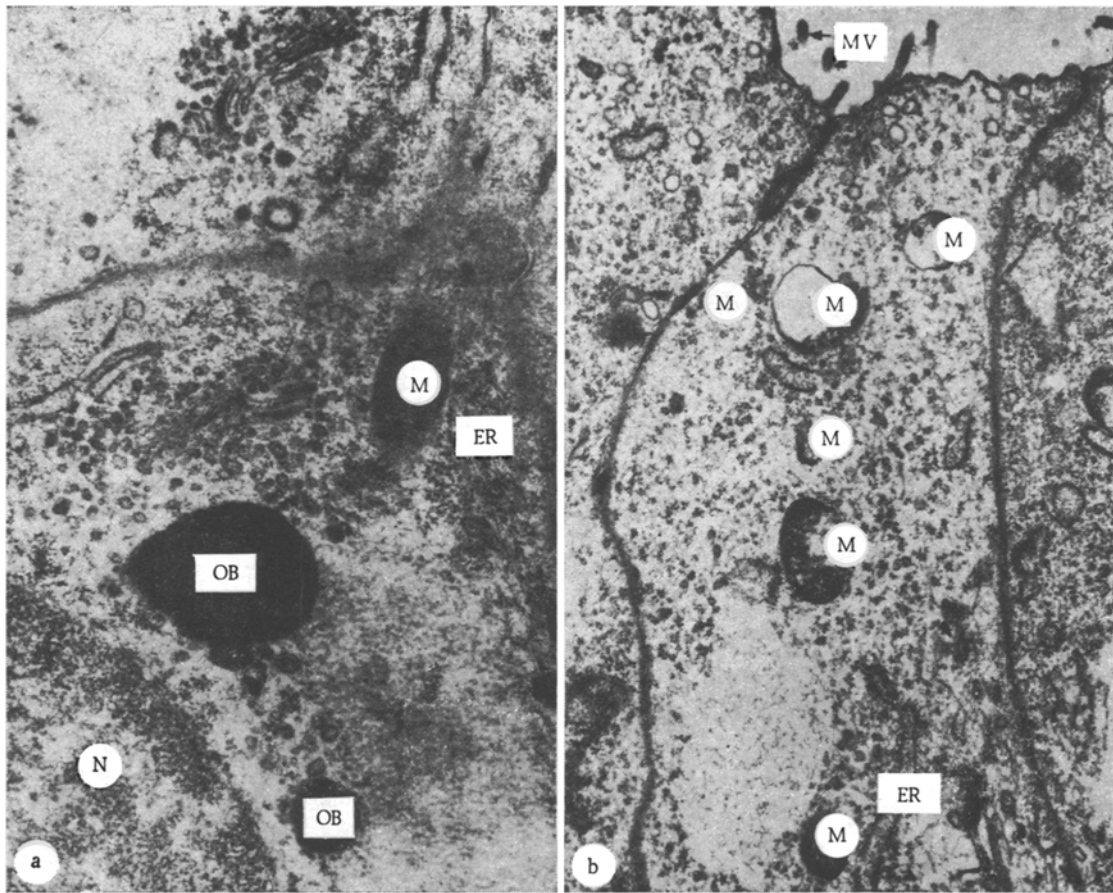


Fig. 1. Epithelium of respiratory anlage on 18th day of fetal development: a) lungs of fetuses of intact rats (8000 \times); M) mitochondria; MV) microvilli; ER) endoplasmic reticulum; N) nucleus. b) Lung of fetus of pneumonectomized rat (2000 \times).

a magnification of 250. By means of a "Videoplan" system, the ratio of the area of the mesenchyme to the area occupied by anlagen of the respiratory portions was determined on the photographs. The number of type II pneumocytes was counted in semithin sections under an immersion system and expressed in promille. Tissue fragments for electron-microscopic study were fixed in a 2.4% solution of glutaraldehyde in 0.1 M cacodylate buffer and postfixed in 1% OsO_4 solution. The material was treated in the usual way and embedded in Epon-Araldite. Semithin sections 1-2 μ thick were cut from each block and stained by the method in [9]. Ultrathin sections were cut on an LKB-8800 Ultratome and examined in a BS-500 electron microscope (Czechoslovakia).

EXPERIMENTAL RESULTS

On the 18th day of fetal development extensive areas of mesenchyme, containing bronchi, blood vessels and anlagen of the respiratory portions, were found in the lungs of fetuses of intact rats. Epithelial cells lining the respiratory anlagen were cubical or, less frequently, cylindrical in shape and 16-20 μ in height. The cells lay on the basement membrane separating the epithelium from the mesenchyme. Nuclei of epithelial cells up to 10 μ in diameter occupied a large part of the cell and contained one or two nucleoli. Mitochondria were round or oval in shape, and possessed an electron-dense matrix with distinct cristae. Cisterns of the rough endoplasmic reticulum were well developed. A large part of the perinuclear zone of the epithelial cells was occupied by concentrations of glycogen granules. The cytoplasm of individual epithelial cells contained one or two osmiophilic lamellar bodies (OB), which were small, electron-dense structures measuring 0.7-0.9 μ , with indistinct lamellae (Fig. 1). Their appearance is evidence that the epithelium of the respiratory portions has started to differentiate. Mesenchymal cells, adjacent to the respiratory anlagen were undifferentiated on the 18th day of fetal development. A characteristic feature of these cells was the presence of homogeneous lipid granules, varying considerably in size and shape. The ratio of the area

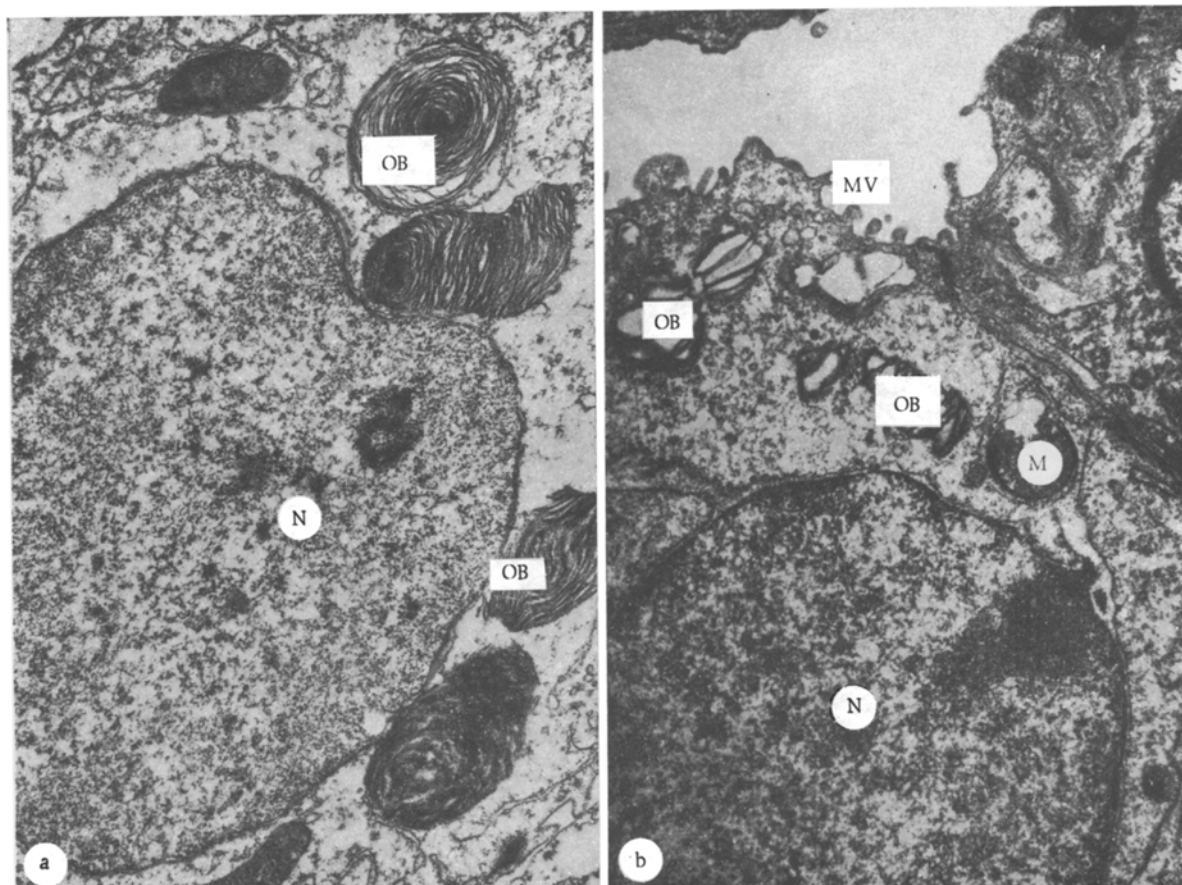


Fig. 2. Epithelium of respiratory anlage on 21st day of fetal development (8000 \times).
Legend as to Fig. 1.

occupied by mesenchyme to the area of the respiratory anlagen on the histological sections was 15.81 ± 1.17 .

The morphological picture observed in histological preparations and semithin sections of the lungs in fetuses of the pneumonectomized rats (experiment) was that characteristic of the fetal lung tissue in the control: small respiratory anlagen, bronchi of various sizes, and blood vessels were distributed among the cells of the mesenchyme. At the ultrastructural level changes typical of edema were observed in the cytoplasm of many cells in the epithelium of the respiratory anlagen. The mitochondria were swollen, with a translucent and vacuolated matrix and with partial destruction of the cristae (Fig. 1b). Cisterns of the endoplasmic reticulum were greatly dilated. In some cells electron-transparent zones of rarefaction of the cytoplasm were observed. The most important fact is that no OB were found in the epithelium of the respiratory anlagen in the experimental series, whereas they were present in the epithelial cells at this time of the investigation in the control. Mesenchymal cells in the experimental series did not differ from those in the lungs of fetuses of intact animals. The ratio between the area occupied by mesenchyme and the area of the epithelium of the respiratory anlagen on the 18th day in the experimental was 19.23 ± 1.22 , which is 1.2 times greater than in the control.

Light-optical investigation of the lungs of 21-day fetuses of intact rats showed that the organ consists of numerous channels and primary alveoli, corresponding to the future alveolar passages and alveoli of the adult lung. In addition, the lung contained bronchi and blood vessels of varied caliber. The spaces in the respiratory anlagen were wider than at the previous time of investigation. The primary alveoli had the appearance of thin-walled sacs lined with simple epithelium, which was flatter than in 18-day fetuses. On electron-microscopic investigation clear differentiation of the epithelium was observed into pneumocytes of types I and II. The type I pneumocytes were flattened in shape, with a large nucleus and characteristic cytoplasmic processes about 10μ long. The type II pneumocytes were larger than the type I pneumocytes, and had microvilli on the apical surface of the cell; while the cytoplasm contained numerous organoids. The mitochondria had an electron-dense matrix and a parallel ar-

rangement of their cristae. The endoplasmic reticulum was well developed with predominance of rough, dilated profiles. The zone occupied by the lamellar complex was large. The number of osmiophilic lamellar bodies in the type II pneumocytes was much greater than at the previous time of investigation, namely 7 to 15 per section through the cell. They measured 1.7-1.9 μ and, as a rule, they were oval in shape with many clearly defined lamellae (Fig. 2a). The ultrastructure of the type II pneumocyte, as we observed it, suggested a cell with high functional activity. The number of type II pneumocytes in the lining of the epithelium of the respiratory anlagen was $644 \pm 53\%$. Cells of the mesenchyme, located near the respiratory anlagen, were mainly undifferentiated, and fibrillary structures could be seen among them in some places. The ratio of the area occupied by the mesenchyme to the area of the respiratory anlagen was less than at the previous time of investigation, namely 1.96 ± 0.27 .

Type I and II pneumocytes were observed in the epithelium of the respiratory anlagen of 21-day fetuses of the pneumonectomized rats, just as in the control. The structure of the type I pneumocytes was indistinguishable from the control. The cytoplasm of most type II pneumocytes, by contrast with the control, was edematous, with low electron density. Destruction and edema, loss of the double outer membrane, focal homogenization of the matrix, and the appearance of vacuoles at one of the poles were observed in the majority of mitochondria (Fig. 2b). Widened profiles predominated in the endoplasmic reticulum. Just as in the control these cells contained osmiophilic lamellar bodies (8-10 per section through the cell). They measured 0.9-1.1 μ and they contained a few lamellae. The number of type II pneumocytes lining the respiratory anlagen was $270 \pm 24\%$, or 2.4 times less than in the control. The ratio of the area occupied by mesenchyme to the area of the respiratory anlagen on the histological sections was 5.78 ± 0.74 in the experiment, which was 3 times greater than in the control ($p < 0.02$).

The lung of the fetuses of pneumonectomized rats is thus characterized by delay in the differentiation of the epithelium of its respiratory anlagen, which is manifested, first, as the later appearance of OB and, second, the smaller number of type II pneumocytes in the lining of the respiratory anlagen before birth. The OB also are less well differentiated than in the control. Electron-microscopic investigation of the fetal lungs in the experimental series revealed edema in many cells of the epithelium of the respiratory anlagen at the times studied, evidence of the depressed functional activity of these cells.

Histological investigation showed that the ratio of the principal structural elements of the organ — the stroma and parenchyma — is disturbed in the lungs of fetuses of pneumonectomized rats. The ratio of the area of mesenchyme to the area occupied by respiratory anlagen was 1.2 times greater in the experiment than in the control on the 18th day of intra-uterine development and 3 times greater on the 21st day. It can be concluded from these observations that the lungs in fetuses of pneumonectomized mothers are functionally less mature.

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