HISTOCHEMISTRY OF LIPIDS IN THE LUNGS

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The participation of the lungs in lipid metabolism has been associated with the function of the lungs as a fat depot [1], although other authorities consider that lipids undergo oxidation in the lungs [11], a process connected with the heat-forming function of these organs [3]. Recently, results have been obtained showing that lipids secreted onto the surface of the alveolar epithelium lower the surface tension in the alveoli, an important factor for stabilization of the air space [4, 12, 15]. Electron microscopic studies of rats have shown that numerous osmiophilic inclusions appear in the granular cells of the lungs two days before birth. In newborn rats, destroyed granular cells can be seen, and in some cases a thin lipid layer is found on the surface of the alveoli [5, 9, 10]. In adult animals lipids are deposited in the large alveolar cells [7], some of which may also be found in the lumen of the alveoli [13]. The nature of these cells has not been settled. In anoxia [14] and in lipidosis [6], deposition of tiny lipid droplets was observed in the desquamated alveolar epithelium.

In the present investigation the distribution of lipids in the lung structures was studied by histochemical methods at different stages of ontogenesis: in the embryonic period – in rat and mouse embryos, and during postfetal development – in various mammals (rats, mice, guinea pigs, dogs, and cats).

EXPERIMENTAL METHOD

Material was fixed in Baker's mixture of 12% neutral formalin. Sections cut to a thickness of 10 μ on a freezing microtome, were stained with Sudan black or, in some cases, with Sudan III. Some material was fixed by Ciaccio's method, embedded in paraffin wax, and cut into sections which were stained with Sudan black. In parallel experiments the material was fixed in Zenker-formol, embedded in paraffin wax, and sections were stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

The anlagen of the lungs in rats were studied from the 15th day of embryonic development. Until the 20th-21st day of embryonic development, i.e., until formation of the primary alveolar sacs, either lipids could not be detected, or they appeared as tiny inclusions, staining with Sudan black. In 21-day fetuses, the epithelium of the primary alveolar sacs contained numerous lipid inclusions, varying in size (Fig. 1). In the young rats in the first days of life, numerous lipid inclusions also appeared in the epithelial cells of the newly formed respiratory segments. In lung sections from 12-day-old animals in some areas the number of inclusions remained as large as before, while in others they were considerably fewer.

Examination of preparations stained with hematoxylin-eosin showed that in some parts the respiratory segments were differentiated to a considerable degree, while in others the walls of the alveolar sacs were comparatively thick, and the septa were correspondingly few. Postfetal development of the lungs in mice [2] and also in rats takes place mainly by progressive centripetal septation of the alveolar sacs. Areas with a low content of lipid inclusions correspond to more highly differentiated respiratory segments. Many lipid inclusions were found in the epithelium of undifferentiated alveolar sacs. By the 21st day of postembryonic development all the respiratory segments were largely differentiated, and no areas with a high content of lipid inclusions could be found. A few tiny lipid inclusions were observed in the alveolar epithelium. However, a few alveolar cells accumulating numerous lipid inclusions in their cytoplasm appeared in the interalveolar septa. With age, these cells became more numerous in the interalveolar septa (Fig. 2). Some could be seen in the lumen of the alveoli. With age many such cells were formed in the

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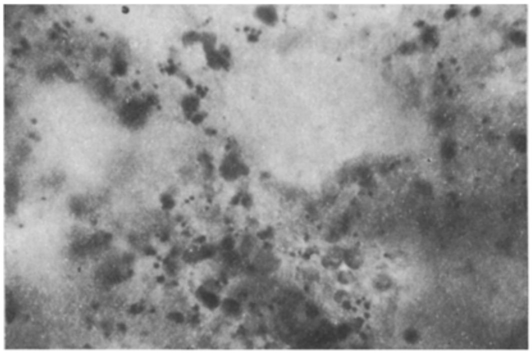

Fig. 1. Lung of a 21-day rat fetus. Lipid inclusions of varying size are visible in the epithelium of the primary alveolar sacs. Fixation with neutral formalin, stained with Sudan black. Objective 60, ocular 10.

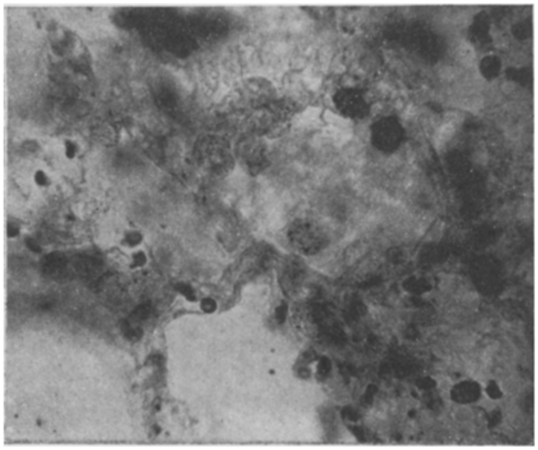

Fig. 2. Lung of a rat aged 4 months. Cells with numerous lipid inclusions are visible in the interalveolar septa. Fixation with neutral formalin, stained with Sudan black. Objective 60, ocular 10.

interalveolar septa of the respiratory segments around the pulmonary veins and bronchi. In old rats alveolar cells with a high content of lipid inclusions were much more numerous. The whole cytoplasm of these cells, which were larger in size, was filled with these inclusions. Most commonly cells of this type were also found in the lumen of the alveoli. A few of them could be clustered together, filling the whole free space of the alveoli. In old rats tiny lipid inclusions also appeared in the epithelium of the small bronchi.

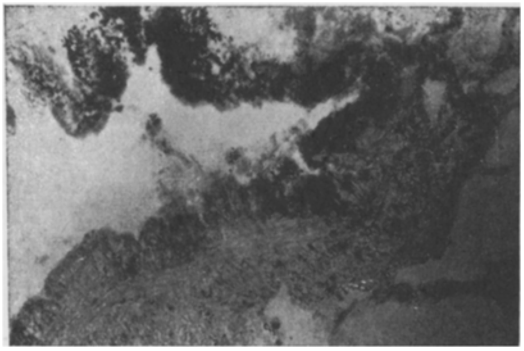

Fig. 3. Lung of a dog aged 8 years. Numerous lipid inclusions visible in epithelium of a terminal bronchiole. Fixation with neutral formalin, stained with Sudan black. Objective 20, ocular 10.

In the mouse fetuses in the last days of embryonic development, just as in the rat fetuses, numerous lipid inclusions were found in the epithelium of the primary alveolar sacs. The pattern of change in the content of lipid inclusions in the postembryonic period was the same as in the rats.

In newborn kittens, puppies, and guinea pigs, lipid inclusions were also found in the epithelium of the interalveolar septa, but in smaller numbers than in newborn rats and mice. More lipid inclusions were found in the kittens and puppies than in guinea pigs. In all newborn animals of this group a few cells with clearly defined borders and numerous lipid inclusions were found in the interalveolar septa. Just as in the rats, with age the number of these cells in the interalveolar septa increased, but in the total mass of cells of the alveolar epithelium the lipid inclusions became fewer in number. In the cats and dogs the content of lipid inclusions in the epithelium of the terminal and alveolar bronchioles gradually increased with age. In the adult cats and dogs the epithelium of some terminal bronchioles contained so many lipid inclusions that they filled the whole cytoplasm. The epithelium of these bronchioles stained with Sudan black (Fig. 3).

The decrease in the lipid content in the total mass of cells of the alveolar epithelium after birth must be associated with the gas-exchange function of this epithelium. A high content of lipid inclusions would inhibit gas exchange. With age, the cells scattered among the alveolar epithelium and accumulating large quantities of lipid inclusions became more numerous in all the animals. These cells should be regarded as derivatives of the alveolar epithelium. The main factor responsible for their formation is the respiratory function, for these cells became more numerous with age.

The experiments showed that the lipid inclusions are mainly phospholipids. This is confirmed by the results of biochemical investigations [8]. The large droplets contained in some cells with numerous lipid inclusions stained well with Sudan III.These cells became more numerous in the old animals. Since Sudan III reveals mainly neutral lipids, it must be assumed that the large drops discovered in some of the cells were neutral lipids, possibly formed as a result of degeneration of the cytoplasm of these cells.

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