apparatus in the cells of the regenerating lung (Figs. 1c and 3a). Scanning electron microscopy showed that the brush cells are in fact capable of secretion (Fig. 2a, b).

The electron-microscopic data thus show that alveolar brush cells can perform several functions: absorptive, contractile,and secretory. The microfibrillary apparatus of the alveolar brush cells contains proteins that differ from the tubulins of microtubules.

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ELECTRON-MICROSCOPIC AND ELECTRON-AUTORADIOGRAPHIC CHARACTERISTICS OF EMBRYONIC LUNG CELLS IN ORGAN CULTURES

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Electron-microscopic and electron-autoradiographic investigations of embryonic mouse lung cells were undertaken during the early stages of organotypical culture. The fate of undifferentiated cells could be traced by electron autoradiography.

KEY WORDS: Electron-microscopic autoradiography; histological differentiation; ultrastructural differentiation.

The relationship between differentiation at the histological (tissue, organ) and subcellular levels, and in which they occur have not yet been adequately studied [1, 6, 8, 9, 11], and this is an obstacle to our understanding of the mechanisms of morphogenesis taking place under physiological (regeneration etc.) and pathological (tumor growth etc.) conditions. Organ cultures of embryonic tissues, in which morphogenetic processes similar to those in vivo are preserved, constitute a convenient model with which to study this problem [3, 5, 7]. The object of the present investigation was to study the degree of differentiation of cells in the developing embryonic lung under conditions when histological differentiation of the tissue had not yet reached the postnatal level.

EXPERIMENTAL METHOD

The lungs of 18-19-day line A mouse embryos were used for organ culture. The technique of organotypical culture was described previously in detail [3]. Organ cultures were investigated 24, 48 and 72 h after the beginning of culture. Thymidine-^{3}H (specific activity 24 mCi/mmole) was added to the organ culture in a dose of 5 mCi/ml medium 24 h after the beginning of culture. The thymidine-^{3}H was washed out of part of the material after 1 h and of the rest after 24 h. Pieces of lung tissue were fixed in 2.5% glutaraldehyde solution in cacodylate buffer, pH 7.4, postfixed in 1% OsO_4 solution, dehydrated, and embedded in Epon 812. Sections 10 μm thick were prepared by the method of Rengol'd et al [4], coated with Ilford L-4 emulsion, and exposed for 14 days at 4°C. After development of the autoradiographs, pieces of tissue containing the label were selectively

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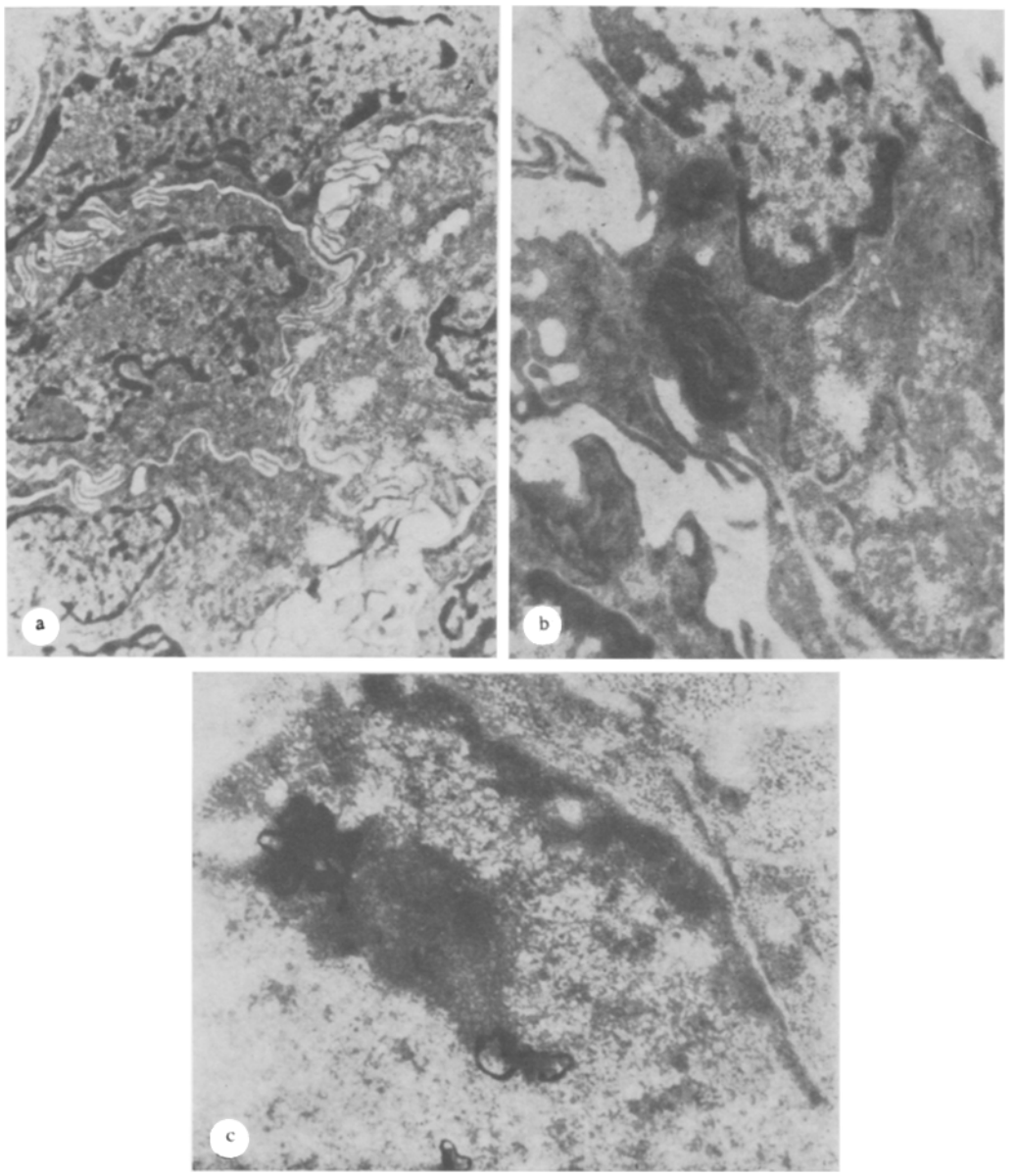

Fig. 1. Electron micrographs of organ culture after 24 h: a) general appearance of undifferentiated cells of organ culture, 7000×; b) fragment of cell with lamellar osmiophilic bodies characteristic of type II pneumocytes in its cytoplasm, 12,000×; c) Fragment of undifferentiated cell with label, 18,000×. Here and in Fig. 2: N) nucleus; LOB) lamellar osmiophilic bodies.

cut out for subsequent electron-autoradiographic investigation. Ultrathin sections 50 nm thick were cut from the selected areas of tissue on the LKB-III Ultratome, coated with Ilford L-4 emulsion, exposed for 60 days at 4°C, and developed in amidol developer recommended by the Photographic Chemical Research Institute and studied in the IEM electron microscope.

EXPERIMENTAL RESULTS

Electron-microscopic investigation of the embryonic lung 24 h after the beginning of organ culture showed that the tissue consisted almost entirely of primitive cells whose ultrastructural organization was such that they could not be classed as any type of cell found in the pulmonary epithelium (Fig. 1a). These were small cells with large, lobular nuclei. The chromatin was distributed diffusely over the nucleus, forming larger concentrations chiefly along the inner nuclear membrane. In some nuclei finely granular nucleoli could be observed. The cytoplasm was extremely poor in ultrastructures but contained solitary small mitochondria, ribosomes, and glycogen granules. The plasma membrane of each cell bordered on the corresponding membranes

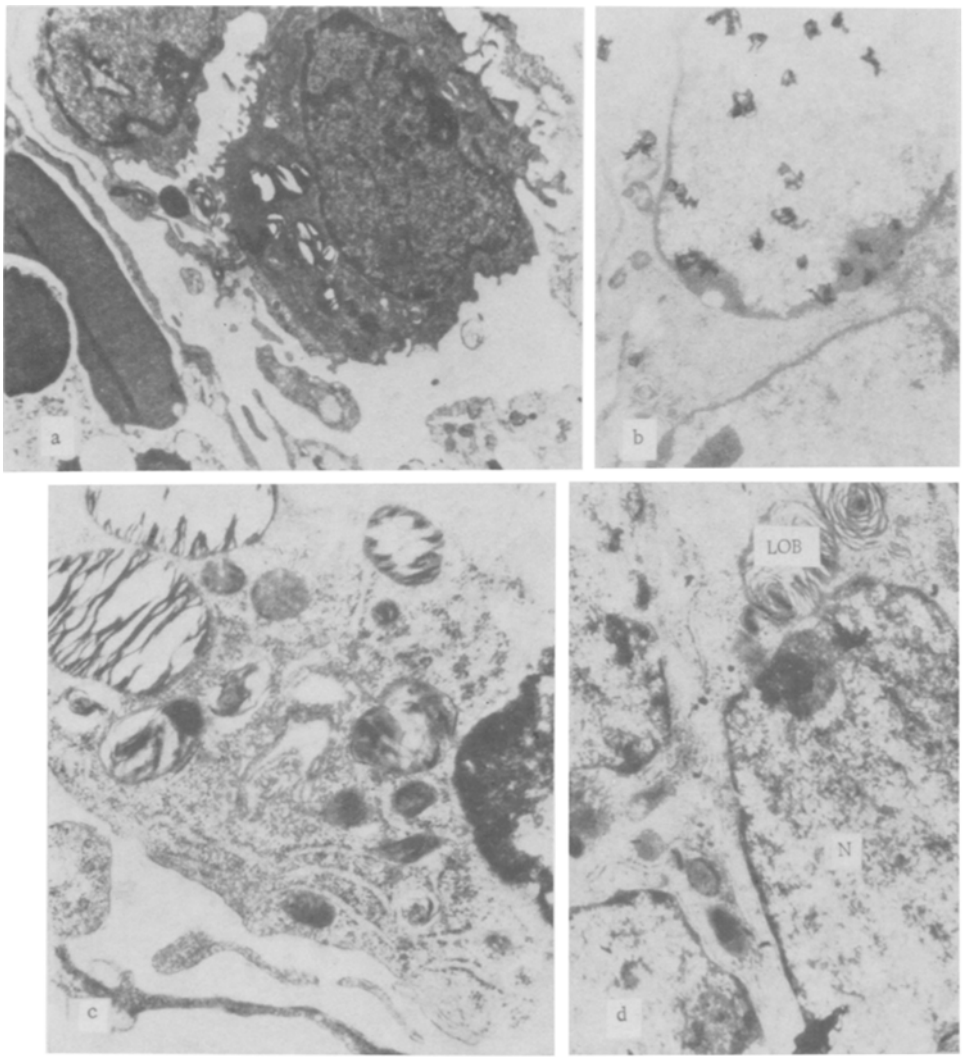

Fig. 2. Electron-micrographs of organ culture after 48 h (a, b) and 72 h (c, d). a) General view of cell differentiating into type II pneumocyte. Lamellar osmiophilic bodies of different densities can be seen in cytoplasm, 7000×; b) undifferentiated cell with intensive labeling above nucleus, 8000×; c) fragment of differentiated type II pneumocyte, 18,000×; d) label above nucleus of type II pneumocyte, 9000 x.

of 3-5 cells. It was undulating and formed junctions of interlocking type. The width of the intercellular space varied. Besides the relatively undifferentiated cells described above, there were also a few cells in whose cytoplasm there were densely packed lamellar osmiophilic bodies characteristic of type II pneumocytes, surrounded by a single membrane (Fig. 1b). Well-marked degenerative changes could be seen in many cells of the explants.

There were very few labeled cells. The labels observed above the nuclei of undifferentiated cells, where it was localized above the nucleoli or the nuclear chromatin (Fig. 1c). The intensity of labeling was low – only two or three grains of silver. No label was presented in cells with ultrastructural features of type II pneumocytes.

After 48 h of organ culture the tissue consisted of loosely arranged cells at different levels of differentiation. Besides undifferentiated cells, others were seen that were differentiated and had specific ultrastructural features. These were cells in whose cytoplasm lamellar osmiophilic bodies of different density could be seen, evidently reflecting differences in their degree of maturity (Fig. 2a), as well as cells whose plasma membrane formed numerous microvilli and cilia with complex structure on the apical part.

Labeled cells were more numerous than at the previous time. Intensive labeling was observed in some undifferentiated cells, which had 15 to 20 grains of silver above the nucleus (Fig. 2b). In some differentiated cells, label also was found above the nuclei, but there was less of it (from 2 to 10 grains of silver).

Sections through the explants after 72 h of organ culture consisted of densely packed cells. Their ultrastructural organization was more complex still. Most of the type II pneumocytes were highly differentiated cells (Fig. 2c), similar in their ultrastructural organization to the corresponding cells in adult animals [2]. Type I pneumocytes, cells with zymogen granules, appeared for the first time in the field of vision at this stage of culture. Cells of the ciliated epithelium had their organelles distributed at the poles.

The number of labeled cells was increased. Most of them were differentiated (type II pneumocytes, ciliated epithelial cells, goblet cells) with a low intensity of labeling, namely 2-7 grains of silver (Fig. 2d).

By electron-autoradiography it was possible to trace the fate of the undifferentiated cells. After pulse labeling 24 h after the beginning of culture, grains of silver were observed above the nuclei only of undifferentiated cells, and the labeling index was considerably lower than that observed by Kury [11] in the developing mouse lung on the 18th-19th day of embryonic development. The reason was probably that in organ culture the tissue adapts itself to its new conditions, and the duration of the presynthetic period is considerably increased in most cells.

Two types of labeled cells were observed after 48 h in culture. These were undifferentiated cells with intensive labeling, constituting the majority, and cells which had begun to differentiate and had evidently taken up the label during division of the labeled precurser cell.

The cell cycle in the pulmonary epithelium on the 19th-20th day of embryogenesis is known to extend over 16.25-17.25 h in peripheral areas and rather more in central zones. On the basis of these figures, since the duration of the cell cycle of embryonic cells in organ culture is unknown, but is evidently a little longer, it can be postulated that some of the undifferentiated DNA-synthesizing cells had succeeded in dividing and starting to differentiate. This hypothesis is supported by the absence of label in differentiated cells on pulse labeling after 24 h in culture, and its appearance in them after 48 h and, in particular, after 72 h in culture, but with a much lower labeling index.

After 72 h in culture, most labeled cells were differentiated, but the intensity of labeling was low.

Comparison of the degree of differentiation at the histological and ultrastructural levels thus shows that these two processes are not synchronized in culture, subcellular differentiation preceded histological to some degree.

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