ORGAN CULTURE OF HUMAN EMBRYONIC LUNG TISSUE

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Organ culture of embryonic lung tissue from 22-24-week human fetuses was carried out. Morphological investigation showed that during culture of human lungs for 30 days the same periods of adaptation, optimal growth, differentiation, and gradual death are observed as in the case of corresponding cultures from rodents. The distinguishing features of organ cultures of embryonic lung tissue from man and rodents are discussed.

KEY WORDS: *organ culture; human embryonic lung tissue.*

For several years the writers have been engaged in organ culture of various organs and tissues. The special features of morphogenesis and survival rate of explants have been studied in relation to the species, line, and age of the donor animal during long-term culture of, in particular, lung tissue [1-4].

This paper describes the results of long-term organotypical culture of human embryonic lung tissue. Such an investigation is essential for assessment of the changes arising during the action of carcinogens on the corresponding organ cultures [5, 6, 8].

EXPERIMENTAL METHOD

The lungs of 22-24-week human fetuses were used for explantation. Culture was carried out by a modified watch glass method, details of which were described earlier [1]. Altogether 644 explants from six fetuses were studied during culture for periods ranging from 3 to 30 days (Table 1).

EXPERIMENTAL RESULTS

The embryonic lung of the 22-24-week human fetus consists of comparatively infrequently branched bronchi and bronchioles, together with alveolar passages lined with cubical and cylindrical epithelium and surrounded by islands of interstitial tissue. The latter consists of loose connective tissue rich in blood vessels and intercellular substance, the predominant cells in which are fibroblasts, endothelium of blood vessels, and reticular cells.

During the first days of culture the explants were almost indistinguishable from the original lung tissue in their morphological structure. On the third day the bronchioles were a little dilated, pycnotic nuclei were distributed uniformly throughout the tissue of the explant, and remains of desquamated cells could be seen in the lumen of some bronchioles and alveolar passages (Fig. 1). In some cases the organotypical structure was disturbed in the center of the cultures and degenerative or even necrotic changes were observed.

On the 7th-10th day some flattening and active desquamation of the epithelium

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TABLE 1. Changes in Organ Cultures of Human Embryonic Lung Tissue with Time

Duration of culture (in days)	Number of cultures	Normal		Degenerative changes		Necrotic changes	
		abs.	%	abs.	%	abs.	%
3	102	82	80,5	13	12,7	7	6,8
6—7	149	64	43,0	34	22,8	51	34,2
9—10	157	55	35,0	41	26,2	61	38,8
13—14	48	26	54,1	9	18,8	13	27,1
17	68	28	41,2	12	17,6	28	41,2
20—21	32	20	62,5	1	3,1	11	34,4
25	52	11	21,1	11	21,1	30	57,7
28—30	36	0		4	11,1	32	88,9

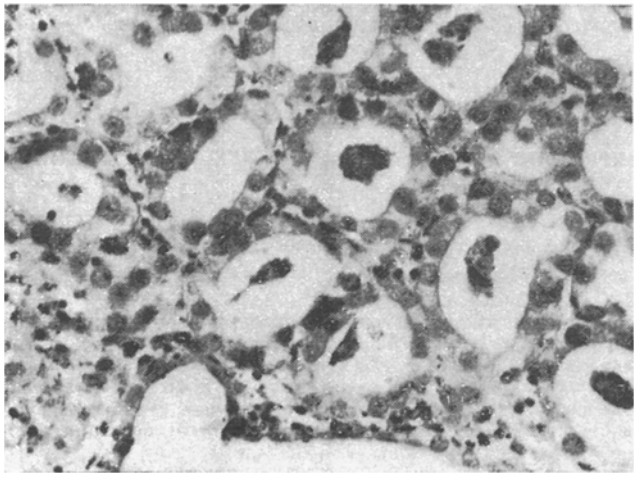

Fig. 1. Human embryonic lung tissue, third day of culture: bronchioles lined with cubical epithelium. Here and in Figs. 2 and 3, magnification 400X.

were observed. As a result, in some bronchioles the epithelial lining was completely absent and detritus was present in their lumen (Fig. 2). The interstitial tissue was much more compact, the vascular endothelium in it had frequently proliferated, and blood cells had escaped from the vessels and infiltrated into the surrounding tissue. Degenerative changes and necrosis were seen more frequently in the center of the explants. At the periphery, however, the tissue was normal and mitoses were frequently seen, especially in the interstitial tissue.

On the 14th day of explantation organization of the primary necrotic masses took place, the epithelium continued to flatten and parts of it had degenerated and desquamated into the lumen of the bronchioles.

By the 17th-21st day of culture the epithelial lining was partly or completely absent in many of the bronchioles examined. Under these circumstances the tissue of the explant consisted of a distinctive connective-tissue skeleton preserving the structure of the lung tissue. The preserved epithelium in most of the bronchioles showed degenerative changes and was desquamated into their lumen. However, often bronchioles without an epithelial lining and others lined with a single-layered, flattened epithelium without evidence of degeneration, and even containing mitoses, were found in the same explant (Fig. 3). Although compared with the first periods of culture the number of primary bronchioles was not increased, and in most of them the epithelium had died, nevertheless their lumen had become wider and more branched and the residual epithelium was a little thickened.

These findings suggest that certain processes of growth and differentiation were taking place in the embryonic lung tissue. They were most marked in the interstitial tissue, the relative area of which had increased considerably, as had the density and maturity of its cellular components, chiefly fibroblasts and vascular endothelium. The latter proliferated as thick, concentric bundles.

Later, by the 25th day, the frequency of the degenerative changes and their intensity increased sharply and the organotypical structure was disturbed. Necrotic changes appeared more and more frequently, causing death of the culture as the experiment continued. The dynamics of survival of organ cultures of human embryonic lung tissue is shown in Table 1.

Comparison of the statistics and morphological picture showed certain regular features. For instance, during the first days intensive death of individual cells or groups of cells, evidently damaged during preparation of the tissue for explantation,

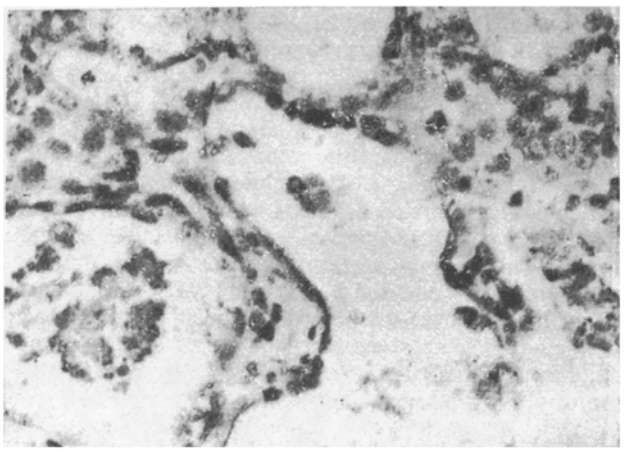

Fig. 2

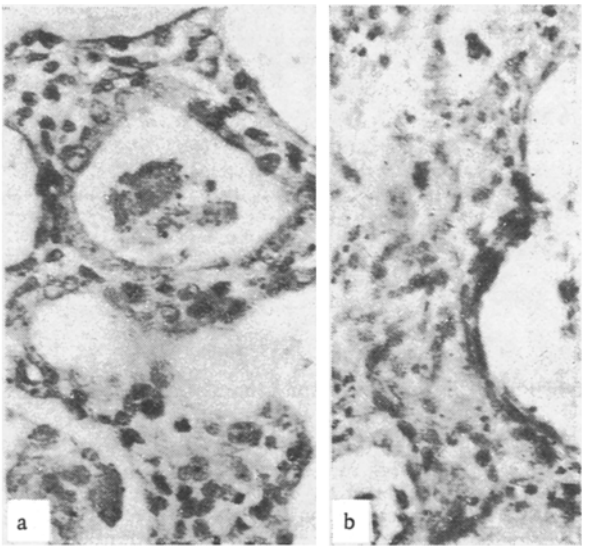

Fig. 3

Fig. 2. Human embryonic lung tissue, 10th day in culture: proliferation of interstitial tissue, desquamation of epithelium.

Fig. 3. Human embryonic lung tissue, 17th day in culture: a) bronchioles with intact epithelium, b) marked proliferation of interstitial tissue, absence of epithelium.

took place. By the third day most of these cells were eliminated into the lumen of the bronchioles and alveolar passages. At this time degenerative changes appeared in the center of the explants, where primary central necroses so characteristic of organ cultures and, as will be seen, caused by the particularly unfavorable conditions (obstruction to diffusion of the nutrient medium, inadequate access of oxygen, and so on), developed later. In the peripheral part of the explants, besides death of individual cells, others regenerated: partially in the epithelium and completely in the connective tissue.

Later, from the 14th to the 21st day, the degenerative processes apparently became stabilized whereas growth and differentiation continued. Later (25th-30th day) the degenerative changes increased again and extensive areas of secondary necrosis developed.

During culture of human embryonic lung tissue the same periods thus were observed (adaptation, optimal growth and differentiation, and gradual decay) as during culture of embryonic lung tissue from rodents. The duration of the individual periods and the total time of culture were about the same [5]. However, there were differences. For instance, growth and differentiation in the corresponding human cultures were observed mainly in connective tissue, but in rodent cultures in the epithelium. These differences may perhaps be connected with the different ages of the human (second third of pregnancy) and rodent (third third of pregnancy) fetuses and with the correspondingly unequal degree of differentiation of the tissue components of the embryonic lung tissue. The absence of true differentiation in cultures of human embryonic lung tissue at this stage of embryogenesis has also been noted by Chesterman and Franks [7].

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