CULTIVATION OF NORMAL AND TUMORAL LUNG TISSUE

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Problems of induction of lung cancer, its histogenesis, as well as the methods for producing experimental models of lung cancer have been widely studied [1-4]. Lasnitski was the first to investigate the effect of carcinogens on lung tissue in organ explants. This author as well as most other investigators had used embryonic tissue, which could be explanted easily [6]. Cultivation of adult tissue is not always successful [7, 8], and to this effect there have been only very few publications.

The purpose of this investigation was to determine the optimal conditions for organ culture of normal and cancerous lung tissue and to study the morphological sturcture of the explants at different periods of cultivation.

EXPERIMENTAL METHOD

Embryonic and adult mouse lungs were studied, as well as lung adenoma produced in mice of strain A, by means of urethane. Urethane was introduced intraperitoneally in 20 mg doses with 3 to 4 day intervals, to a total of 100 mg. Numerous lung adenomas were noted in three months' time.

The organs were explanted onto the surface of tissue paper, floating on nutrient medium, according to the method of Chen [5]. The nutrient medium consisted of 10-11 days old chick embryo extract, 1 : 2 in normal saline – 25%, calf serum – 25%, and medium 199-50%. Penicillin in the amount of 100 units/ml of the medium was added in order to prevent bacterial contamination.

Fragments of lung up to 1-2 mm long were cultivated in watch glasses on top of tissue paper which was treated according to the method of Shaffer. The paper was passed consecutively through two changes of ether, and through two changes of absolute alcohol (1½ to 2 hours in each). It was then thoroughly washed in distilled water and left overnight in water. After it was dried, the paper was autoclaved at 160°C for 1½ hours. Four watch glasses, each of which held 4-5 tissue fragments, were placed in a petri dish. Filter paper moistened with sterile distilled water was placed in the bottom of the petri dishes. Culture vessels were placed under a glass hood and kept in an incubator at 35°C. Lung explants were cultured for 3-10 days. Cultures were transferred every 3 days. The explants were fixed in Carnoy's fluid, embedded in paraffin and stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

Our experiments have shown that different conditions were necessary for the successful cultivation of embryonic and adult mouse lung tissue. The adult lung was more difficult to culture than the embryonic lung and lung adenoma. In order to retain the normal morphological structure in the explants of the adult lunt it was necessary to add glucose to the medium (2-3 drops of 40% glucose solution to 10 ml of medium 199), and to add CO_2 to the surrounding atmosphere. Lung adenomas were successfully cultured under the same conditions as were embryonic lung explants.

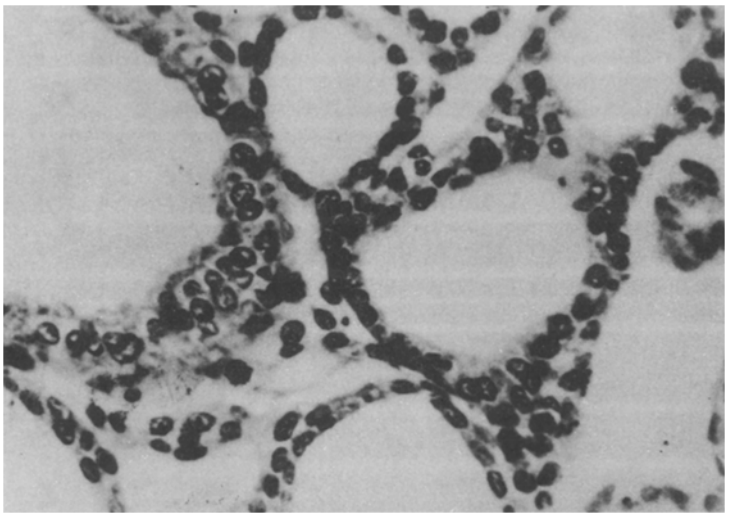

Fig. 1. Embryonic mouse lung in organ culture. 3 day growth. Photomicrograph 500x.

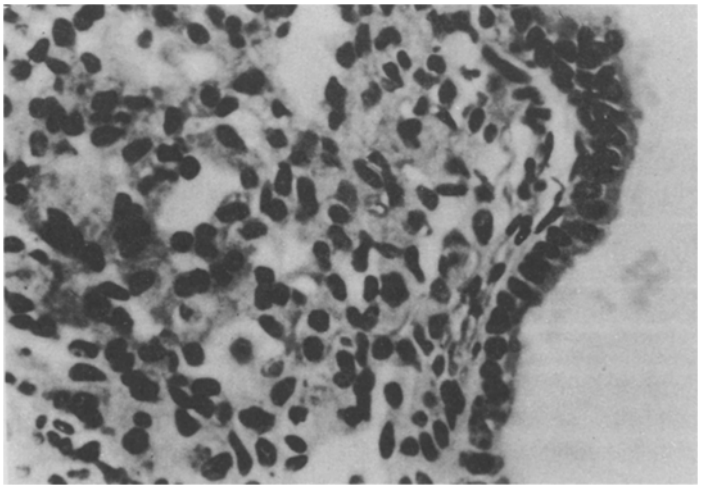

Fig. 2. Adult mouse lung in organ culture. 10 days growth. Photomicrograph 500x.

A morphological study of 3 day old cultures of the embryonic lung has shown that the explants consisted mainly of lung alveoli lined with cuboidal epithelium. There was a relatively large number of bronchioles lined with a single layer of cylindrical epithelial cells. The cells in the alveolar epithelium were the same in size and shape and possessed a homogeneous cytoplasm and a small hyperchromatic nucleus. Individual connective tissue fibroblast-type cells were seen between the alveoli. Mitotic figures were seen most often in the bronchial epithelium. There was necrosis within the explants. The above structure of the organ cultures was similar to that of the embryonic mouse lung (Fig. 1).

The structure of explants of the normal lung cultured for 10 days was somewhat different. During this period there formed a two-layered capsule around the explant: the outer layer consisted of fibroblasts and the inner of cuboidal epithelium.

In some explants the capsules consisted only of fibroblasts. The epithelium in the alveoli became flattened and the epithelial cells in the bronchioles became distributed in several rows. The bronchioles became wider and a large number of fibroblasts was seen around them. Mitotic figures were seen. Explants of adult mouse lung tissue

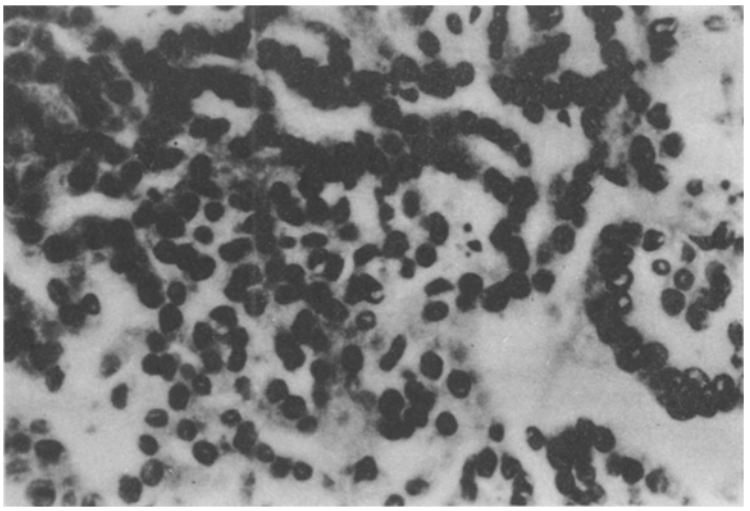

Fig. 3. Mouse lung adenoma in organ culture. 3 day growth. Photomicrograph 500×.

on the third day of cultivation consisted of alveoli lined with epithelium. The epithelial cells were roundish and had a light staining nucleus. In some explants there were bronchi lined with cylindrical epithelium. Connective tissue proliferated around the bronchi. Mitotic figures were only rarely observed.

Capsules, consisting mainly of fibroblasts, were formed around explants after 10 days of cultivation. Alveoli were more compact, and the bronchial epithelium consisted of several rows of cylindrical cells. Mitoses were rare (Fig. 2).

Three day old explants of the mouse lung adenoma, as well as the original tumors, consisted of homogeneous compact tubular formations, lined with cuboidal epithelium. The cells were large, similar in size to each other and had a small hyperchromic nucleus. Separate fibroblasts were seen around the periphery of the explants. Mitotic figures were rare (Fig. 3).

In 10-day old cultures of lung adenoma the cells were less compact. There were glandular irregularly branched structures, lined with polymorphic cells with large nuclei, which were surrounded by a narrow band of cytoplasm. In some areas the cells were distributed in several rows. Mitoses were seen rarely.

The above data have shown that the method of organ explantation permits the cultivation of tissues without disrupting the basic morphological structure. We were able to culture embryonic as well as adult mouse lung tissues.

Conditions for cultivation of embryonic and adult mouse tissues and of cancer tissue differ from each other. Experiments have shown that the growth potential of lung adenoma is similar to that of the embryonic tissue. Adult lung tissue is more fastidious in cultivation, and in order to retain its normal structure it requires additional factors, such as glucose and CO_2.

The method of organ culture allows a study of early changes arising in tissues as a result of the addition of different agents to the culture medium.

The above indicates that this method can be used for the study of different processes of carcinogenesis, chemotherapy, hormonotherapy, etc.

SUMMARY

The author describes a method of culturing the organs (the lung of a mouse's embryo, the lung of an adult mouse and of adenoma), offering a possibility of retention in the explant of the main morphological structure and the usual tissue interrelations. The method allows detailed study of peculiarities attending morphological tissue structure at different culture periods and of the effect of various factors on the organs.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.