ORGAN CULTURES OF THE RESPIRATORY TRACT OF THE HUMAN EMBRYO

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Organ cultures of the trachea remained viable for 2 months, and those of the nasal epithelium and lung tissue for up to 1.5 months. The explants remained susceptible to influenza virus throughout the period of cultivation.

Organ cultures have advantages over monolayer cell cultures: they retain their initial tissue differentiation and their cell relationships, and in some cases even the specialized functions of the tissues. It is no accident, therefore that organ cultures of the respiratory tract are a susceptible material for certain respiratory viruses [4], and they are successfully employed for the isolation of new respiratory viruses [2,3].

The suggestion has been made that some respiratory viruses can propagate only in differentiated ciliary epithelial cells [3].

It is interesting to study certain problems, such as the behavior of vaccine and pathogenic strains of influenza virus in differentiated cells of the respiratory tract, the possibility of passage of viruses to strengthen the immunogenicity of vaccine strains, the mechanisms of cellular immunity, and so on, with the use of organ cultures of the respiratory tract of the human embryo, tissues toward which these viruses exhibit the greatest tropism under natural conditions in the host's body.

However, before turning to the study of these problems it was necessary to test the method of multiple organ cultivation, suggested by Luriya [1] for growth of tissues of the respiratory tract of the human embryo.

In the investigation described below, the maximum period of cultivation of organ cultures was studied, and the morphology of organ cultures of the nasal epithelium, trachea, and lungs was investigated at different periods of explantation. The ability of influenza virus to propagate in these cultures also was examined.

EXPERIMENTAL METHOD

Organ cultures of the nasal epithelium, trachea, and lungs of 5-6-month fetuses (obtained from a maternity home after therapeutic abortion) were used for the experiments. The nasal epithelium, trachea, and lungs were removed under sterile conditions and placed in Petri dishes, after which they were cut up with scissors into pieces measuring 0.5 × 2 mm and bathed in Hanks's solution with antibiotics. The explants were cultivated on HA millipore filters (pore size 0.45 μ).

As far as possible the pieces were placed so that the epithelial surface faced upward. The nutrient medium consisted of 80% medium No. 199 and 20% embryonic extract. For each 1 ml of medium, 4 mg glucose, 10 mg vitamin A, and 100 units each of penicillin and streptomycin were added. Type A2 influenza virus was introduced into each dish with explants in a dose of 10^5-10^6 ID_{50}. Contact with the virus continued for 3 h in an incubator, after which the pieces were washed to remove virus and immersed in medium. The medium was changed every 48-72 h. Before the medium was changed, culture fluid was taken from each dish in which the tissue fragments had been infected with virus, some of the explants were ground in a

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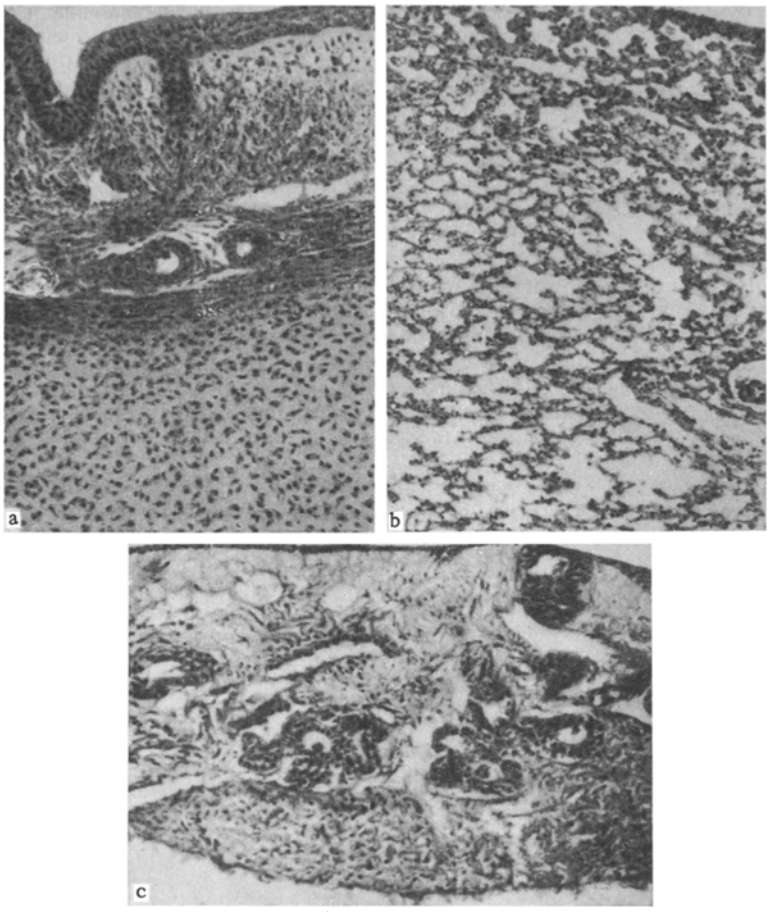

Fig. 1. Organ culture of tissues of a human fetus: a) culture of trachea, 5 days after explantation; b) culture of lung tissue, 14 days after explantation; c) culture of nasal epithelium, 15 days after explantation. Fixation with alcohol-formol, stained with hematoxylin-eosin, 630×.

mortar with Hanks's solution, and the suspension was kept in a refrigerator at −20° until the end of the experiment. All specimens were titrated simultaneously. Serial 1 : 10 dilutions were prepared in medium No. 199. Chick embryos were then infected via the allantoic cavity.

Some of the cultures were fixed with alcohol-formol. At each time 5-10 fragments were taken. The fixed fragments were separated from the filters, embedded in paraffin wax, cut into sections (5-8 μ) on a microtome, and stained with hematoxylin and eosin. Total preparations were made from the filters and these also were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

The histological picture of a section through the trachea of a human fetus in organ cultures after explantation for 5 days is shown in Fig. 1a. It is clear from this figure that no pathological changes were present in the tracheal tissue. The epithelium was stratified and well-differentiated. Many glands were preserved in the underlying connective tissue. Blood cells were packed tightly in the vessels (stasis). Cartilage, still in its normal state, could be seen. At this same time, aggregations of epithelioid cells, arranged in a single layer, were visible on the filter, forming circular structures; the nuclei were round and varied in size, with clearly distinguishable nucleoli.

All elements of the trachea were preserved 15 and 30 days after explantation. Normal epithelium was visible on the surface, and above it cell debris. At these same times cells similar to those described above were observed on the filters. Evidence of the viability of the cultured cells was given by the fact that numerous mitoses were present in them.

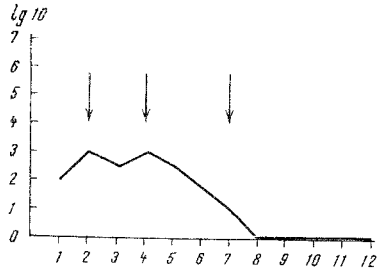

Fig. 2. Propagation of influenza virus A2 M-16 in organ culture of the trachea of a human embryo. Abscissa, titer of infectivity (ID_{50}); ordinate, day of observation; days when medium changed indicated by arrows.

TABLE 1. Propagation of Strains of Influenza Virus A2 M-16 in Organ Culture of Human Embryonic Lung

Strain of virus	Residual virulence to man	Titer of virus		
		day of observation		
		2nd	5th	14th
A2 21/65	–	10^4	10^4	10^3
A2 M 16/25	–	10^4	10^3	–
A2 Smol./65	–	10^2	10^3	10^3
A2 England/64	–	10^4	10^3	10
A2 133/66	–	10^3	–	–
A2 101/59	–	10^4	10^3	10^4
A2 971/59	+	10^5	10^3	10^4
A2 709/59	+	10^4	10^4	10^5
A2 717/59	+	10^4	10^4	10^3
A2 Hong Kong/69	+	10^5	10^4	10^4

The histological picture of sections through the nasal epithelium of a human fetus 15 days after the beginning of cultivation is shown in Fig. 1c. The structure of the nasal epithelium is normal. The epithelial cells were arranged in a single layer. Numerous glands were visible in the underlying connective tissue. One or two isolated collections of fibroblast-like cells were found on the filter 15 days after explantation, with small groups of epithelioid cells here and there among them.

The typical picture of lung tissue remained in the 14-day organ culture of the lung. The alveoli were clearly visible. The sections of the bronchi in the specimen were lined with cubical epithelium. Blood vessels containing collections of blood cells in their lumen were clearly seen. The interstices were filled with connective tissue rich in cells. At this same time the structure on the filter was one of regularly oriented cells with groups of epithelioid cells and isolated connective-tissue cells.

In these experiments the organ cultures of the trachea remained viable for 2 months (period of observation), and the nasal epithelium and lung tissue remained viable for up to 1.5 months.

Besides the morphological investigations, an analysis was made of propagation of influenza virus in organ cultures of the nasal epithelium and trachea.

The explants were infected at the time of explantation and also 7, 14, and 35 days after the start of cultivation. The results showed that tissue variant M-16 of influenza virus A2 proliferated in organ cultures of the nasal epithelium and trachea for 8-10 days (Fig. 2).

The explants retained their susceptibility to influenza virus throughout the period of cultivation.

An attempt was also made to discover whether influenza viruses can multiply in the lung tissues of a human embryo. These results are given in Table 1. In lung tissue explants a high level of propagation of strains of influenza virus A2, both possessing and not possessing residual virulence to man, was obtained.

It was also shown that influenza virus A2 can readily be subcultured through organ cultures of nasal epithelium, trachea, and lungs of a human embryo, and this fact can be utilized to obtain vaccine strains of influenza virus.

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