USE OF THE ORGAN CULTURE METHOD TO STUDY THE COMBINED ACTION OF URETHANE AND INFLUENZA VIRUS ON THE LUNGS OF THE HUMAN EMBRYO

A. F. Frolov, A. M. Shcherbinskaya, and N. E. Botsman

UDC 612.648'215.3-06:[615.277.4+576.858.75]-083.23

The combined action of the chemical carcinogen urethane and of influenza virus on human embryonic lung tissue was studied by the organ cultivation method. The combined action of these factors leads to intensive proliferation of the tissue components, to the appearance of signs of cellular anaplasia, and to the formation of polyp-like structures projecting into the lumen of the bronchi.

In previous investigations the writers showed how the combined action of the chemical carcinogen urethane and of influenza virus could be studied on organ cultures of mouse embryonic lungs [3]. By this means neoplastic transformation of the lung cells was obtained, and this was confirmed by the development of malignant neoplasms of the cytoblastoma type from pieces of transformed lung tissue implanted into syngeneic mice.

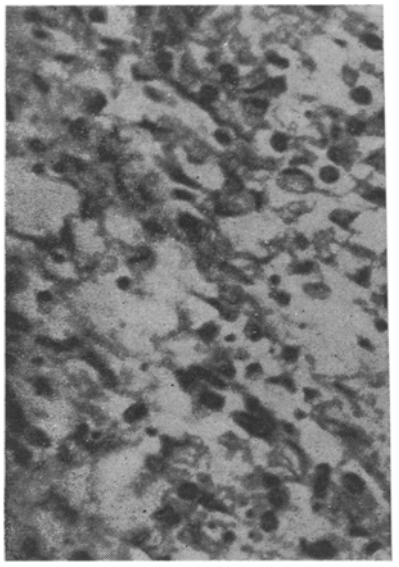

Fig. 1. Organ culture of lung. Control, 12th day of experiment. Degenerative changes in lung tissue. Here and in Figs. 2 and 3: hematoxylin-eosin, 90×.

In the present investigation the action of urethane and influenza virus was studied on organ cultures of human embryonic lung.

EXPERIMENTAL METHOD

Urethane (Ethyl Carbamate). Batch No. 13978, giving all the characteristic reactions for authenticity and purity of this compound, was used as a 2% solution in physiological saline.

Influenza virus. Type A2 influenza virus, strain No. 2226, was used after purification to remove components of the allantoic fluid by the absorption-elution method [2]; infective titer $10^5 EID_{50}$, titer in the passive hemagglutination test 1:1280.

Organ Culture. Organ cultures were prepared from lungs obtained from embryos aged 7-9 weeks. Washed pieces of the lungs were placed on cigarette paper rafts, freely floating on the surface of the nutrient medium [1], which consisted of two parts medium No. 199, one part calf serum, one part embryonic extract, and antibiotics. The medium was changed every 6 days.

Laboratory of Virus Etiology of Tumors, Kiev Research Institute of Epidemiology, Microbiology, and Parasitology. (Presented by Academician of the Academy of Medical Sciences of the USSR L. V. Gromashevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 1, pp. 90-93, January, 1972. Original article submitted October 13, 1970.

TABLE 1. Results of a Study of Histological Sections from Organ Cultures of Human Embryonic Lungs

Experimental condition	Time of observations (days)	No. of sections	Character of changes: necrotic +	++	+++	total	proliferative +	++	+++	total	metaplasia +	++
Control	6	20/20	12	—	1	13	11	2	—	13	—	—
	12	10/20	5	3	—	8	6	4	—	10	—	—
	15	10/10	—	5	5	10	4	—	—	4	—	—
Urethane	6	13/16	3	—	—	3	—	7	3	10	—	—
	12	15/15	—	10	5	15	11	—	—	11	—	—
	15	10/12	—	2	8	10	—	—	—	—	—	—
Virus	6	14/17	3	—	—	3	6	8	—	14	—	—
	12	19/25	6	—	—	6	5	4	9	18	3	—
	15	10/13	7	3	—	10	1	6	3	10	2	—
Urethane + virus	6	19/34	1	—	—	1	—	3	16	19	12	1
	12	19/20	3	1	—	4	1	8	10	19	5	5
	15	10/12	6	1	—	7	—	4	6	10	4	—

Note. Numerator gives number of sections examined histologically; denominator gives total number of explants.

*Results expressed as + signs using the scheme of evaluation proposed by Röller et al. [4].

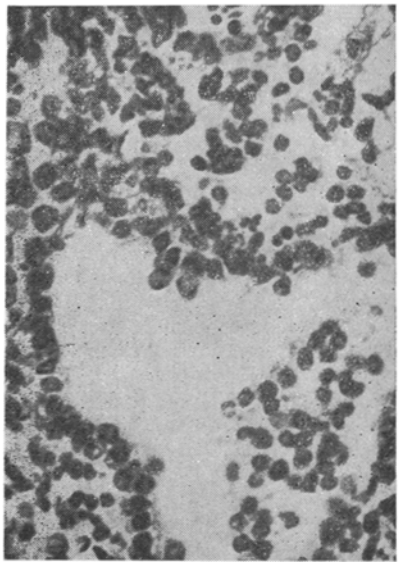

Fig. 2

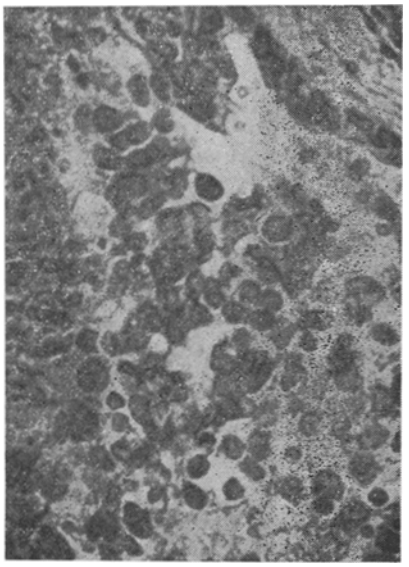

Fig. 3

Fig. 2. Organ culture of lung treated with urethane and influenza virus. Polyp-like proliferation of cells projecting into lumen of bronchus seen in bottom right-hand corner. 12th day of experiment.

Fig. 3. Fragment of organ culture of lung treated with urethane and influenza virus, 12th day of cultivation. Intensive proliferation of alveolocytes and disturbance of alveolar structure of the lung (400×).

Conduct of the Experiment. Experiments were carried out on cultures of four types. 1) Control experiments to test explants from lungs subjected to no treatment; 2) pieces of lungs were placed for 24 h in 2% urethane solution, after which they were washed and transferred to rafts for cultivation; 3) lung embryos were placed for 2 h in virus-containing fluid with a titer of 1:1280; after adsorption (at 25°C) the

pieces were washed and transferred to rafts for cultivation; 4) explants kept in urethane solution for 24 h were placed in virus-containing fluid, and after adsorption they were washed and transferred to rafts for cultivation.

On the 6th, 12th, and 15th days of the experiment explants were taken for histological examination. The material was fixed in formalin, embedded in paraffin wax, and sections were stained with hematoxylin and eosin, and then examined in the MBI-6 light microscope under magnifications of 70, 280, and 630×.

EXPERIMENTAL RESULTS

The principal results are given in Table 1. In the control explants weak, diffuse proliferation of the cells was combined with marked necrotic changes. After the first week of cultivation both the relative number of necrotic foci and the severity of the necrotic changes were increased. Evidence of proliferation was observed (by the 15th day) only at the periphery of the explants: slight thickening of the alveolar septa; narrowing of the lumen of the alveoli and bronchioles; changes in shape of the alveolocytes which became fusiform (Fig. 1).

Intensification of proliferation was observed in the early stages (6th day) of cultivation of the explants treated with urethane, as shown by the formation of discrete but indistinctly outlined foci of hyperplasia, consisting of cells of uniform pattern with oval or round, often hyperchromic, nuclei. However, organ cultures treated with urethane degenerated rapidly; after the 12th day necrotic changes identical in character with those in the control were observed in the sections.

Infection of the human lung explants with influenza virus was followed by rapid proliferation of the cells. Foci of proliferating alveolocytes with hyperchromic nuclei formed in the lung tissues. The normal structure of the lung tissue was completely disturbed, and the lumen of the alveoli and bronchioles was filled with cells. Abnormal mitoses were observed in individual foci. Degenerative changes were absent, even in the late stages (15th day) of cultivation.

The combined action of urethane and influenza virus led to the development of more severe hyperplasia: the interalveolar septa were greatly increased in thickness, and proliferating alveolocytes completely filled the lumen of the alveoli. Polyp-like structures consisting of large, polymorphic cells were formed in the large bronchi into the lumen of which they projected (Fig. 2). This combined action of urethane and virus led to polymorphism and atypism of the cells, the changes in their staining properties, and to the appearance of abnormal mitoses which, to some extent, can be regarded as a manifestation of metaplasia (Fig. 3).

The results indicate that combined action of the carcinogen and influenza virus during organ cultivation leads to the appearance of morphological signs of transformation. These consist of polymorphism of the cells, hyperchromia of their nuclei, multiple (including abnormal) mitoses, and the formation of polyp-like structures in the lumina of the large bronchi.

This potentiation of the action of urethane can evidently be attributed to the fact that under the conditions used in these experiments the influenza virus acted as a stimulant of proliferation. This action was aided by the abortive type of infection taking place in the organ culture and also by the relative virulence of the virus studied. Without causing death of the cells, the influenza virus had a preferential effect on their differentiation and development.

LITERATURE CITED

1. R. Kh. Adil'gireeva, Byull. Éksperim. Biol. i Med., No. 4, 115 (1964).
2. A. S. Gorbunova (Editor), Textbook of Laboratory Diagnosis of Influenza and of Para-influenzal and Adenovirus Diseases [in Russian], Moscow (1960), p. 57.
3. A. S. Frolov, A. M. Shcherbinskaya, and N. E. Botsman, Neoplasma (Bratislava), 17, 103 (1970).
4. M. Röller, S. Owen, and C. Heidelberger, Cancer Res., 26, 626 (1966).