HISTO-AUTORADIOGRAPHIC INVESTIGATION OF DISTRIBUTION OF INFLUENZA A VIRUS IN RESPIRATORY ORGANS OF ALBINO MICE

Kh. Zh. Zhumatov, D. S. Arkhangel'skii, Yu. I. Savchenkov, M. A. Arykova, and V. T. Kuzevanov

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After intranasal infection of albino mice with influenza A virus labeled with P^{32}, it was found to be irregularly distributed as conglomerates in the respiratory organs. Adsorption and penetration of the virus into epithelium of the tracheal and bronchial mucous membrane were observed 15-30 min after infection, and simultaneously with this, the alveoli were cleared of virus particles.

Radioactive isotopes have been used to study penetration of viruses into cells and the mechanism of their interaction with cellular structures in monolayer cultures [3-5, 7, 10-13, 15]. The early cycle of ontogenesis of Myxovirus influenzae and of certain other P^{32}-labeled viruses has also been studied after infection of chick embryos [9, 16]. Histo-autoradiographic investigation of experimental influenza in warm-blooded animals, differing in their susceptibility to the virus responsible for this infection, is the most interesting aspect of this problem. To understand the relative low of the virus itself in the pathogenesis of influenzal infection, the pathways of its progress and reproduction in the respiratory system of the intact organism must be studied. In one investigation described in the literature, I^{131}-labeled influenzal γ globulin was used to detect specific antigens in cells of the nasal mucous membrane of patients with influenza and also in impression films taken from the mucous membrane of the trachea and lung of mice infected with influenza virus [8].

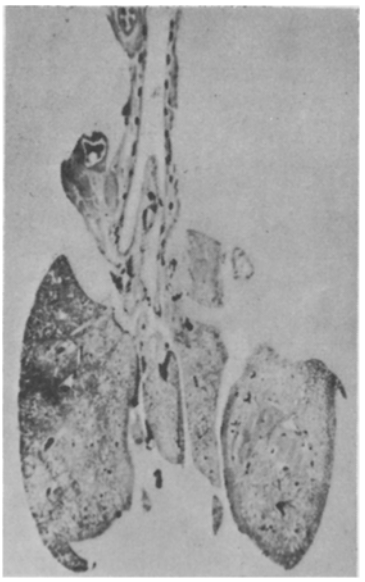

Fig. 1. General appearance of frontal section through respiratory organs of albino mouse infected with P^{32}-labeled influenza virus. Here and in Figs. 2 and 3, staining by azure-eosin. 20 ×.

In the present investigation the distribution of influenza virus in the tissues of the respiratory tract was studied in albino mice by the use of the isotope P^{32}.

EXPERIMENTAL METHOD

Influenza virus of type A, strain Alma-Ata 770/64, was grown in the allantoic cavity of 10-day chick embryos into which a solution of radioactive phosphorus ($Na_2HP^{32}O_4$) with a total activity of 560 μCi per embryo was injected simultaneously (incubation for 48 h). Radiometric measurements were made on a B-2 apparatus with an MST-17 counter. Labeled virus was concentrated and purified twice by adsorption and elution on formalinized hen's erythrocytes.

Institute of Microbiology and Virology, Academy of Sciences of the Kazakh SSR. Scientific-Research Institute of Indigenous Pathology, Ministry of Health of the Kazakh SSR, Alma-Ata. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 10, pp. 27-30, October, 1970. Original article submitted May 23, 1969.

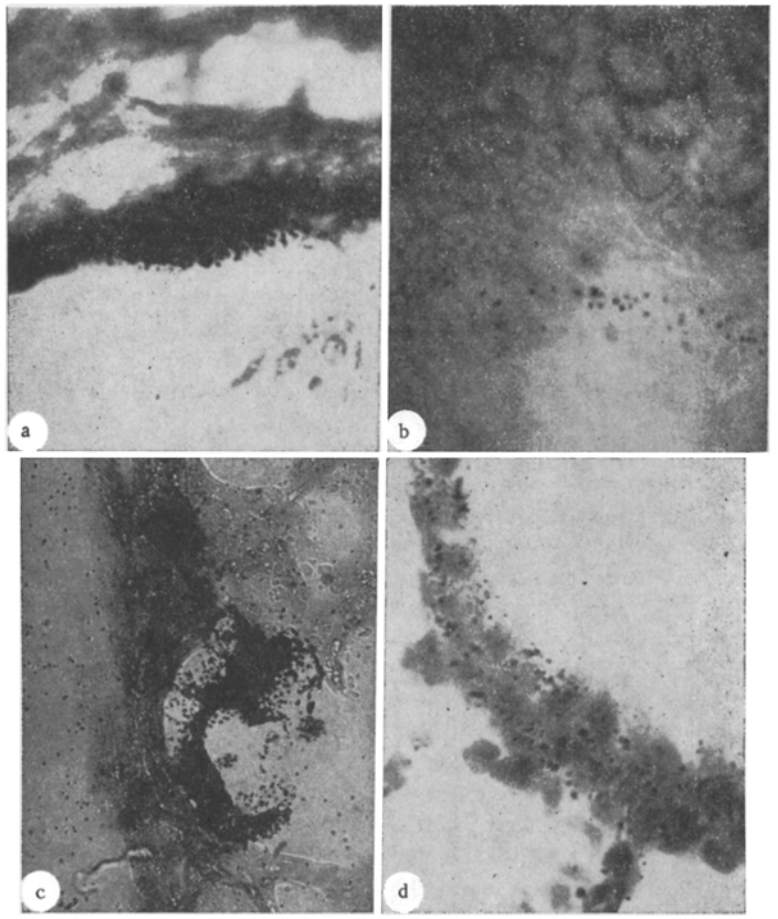

Fig. 2. Area of section through trachea (a, b) and bronchus (c, d) of albino mouse: a, c) immediately after infection (300 ×); b, d) 15 min after contact with virus (600 ×).

The index of purity of the resulting product was absence of radioactivity of the phosphate buffer against which it was dialyzed. The resulting eluate had a hemagglutinating titer of 1:2048, log EID_{50} = 8.23/0.2 ml, and the lethal dose for mice was log LD_{50} = 3.7/0.05 ml. The eluate, with an activity of 8000 pulses/min in a volume of 0.1 ml, was injected in a dose of 0.05 ml intranasally into albino mice weighing 10-12 g. Albino mice receiving P^{32} solution in the same volume and with the same radioactivity acted as the control. The animals were sacrificed immediately after infection and after intervals of 15, 30, and 60 min. The trachea, bronchi, and lungs, removed in continuity, were treated by Santa-Maria's method [14] with certain modifications in order to speed up the fixation and embedding of the sections in paraffin wax in order to allow for the halflife period of radioactive phosphorus (14.3 days). Frontal and sagittal histological sections were obtained from the tissues of the respiratory organs (5-7 μ in thickness), both as total preparations (Fig. 1) and as sections from individual segments. A backing of gelatin, chrome alum and ethanol was applied to the dewaxed sections, after which type R nuclear photographic emulsion was poured over them. The duration of exposure was 6-10 days. After treatment with amidol developer and fixation with sodium hyposulfite, the sections were stained with azure-eosin by Romanovsky's method and examined under the microscope. Photomicrographs were made with the MFN-7 attachment (oil immersion 60 ×, ocular 5 or 10 ×).

RESULTS

Examination of histo-autoradiographs of the respiratory organs of the albino mice showed that immediately after infection the virus was present in the lumen of the trachea, bronchi, and some alveoli as diffuse granules or massive conglomerates.

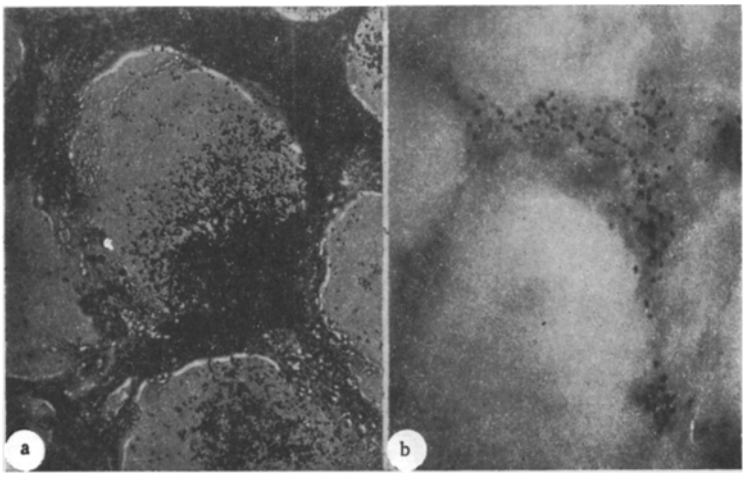

Fig. 3. Area of section through albino mouse lung: a) immediately after infection (300 ×); b) 30 min after contact with the virus (600 ×).

One segment of the trachea is photographed in Fig. 2a, showing clearly defined tracks on its inner surface. Individual tags can be seen above the tracheal tissues, evidently where not all the virus has been adsorbed on to the epithelium. After 15 min the virus is localized in the region of the epithelium lining the middle part of the trachea as separate tracks (Fig. 2b). After 30 min the virus was found in deeper layers of the trachea, and this was particularly obvious 60 min after infection. Judging from the number of tracks, most virus was in the tissues or intercellular spaces.

A histo-autoradiograph of a section through the bronchi of an albino mouse sacrificed immediately after infection with the virus is shown in Fig. 2c. A large collection of virus is visible in the lumen of some of the bronchi, in the form of a conglomerate composed of confluent tracks. The picture was changed somewhat after 15 min: tracks indicated the presence of virus not only in the lumen, but also above the tissues of most bronchi, so that some redistribution of the virus among the bronchial system as a whole had evidently taken place. Penetration of virus into the bronchial epithelium from the periphery and also from the basal layer of cells was visible 30 min after infection (Fig. 2d).

Examination of sections from the lung tissues showed that the virus was located initially in the alveoli as massive conglomerates, but that only a few alveoli were so affected. An area of lung tissue in which the alveoli are irregularly filled with labeled virus is shown in Fig. 3a. A small number of tracks are visible after 15 min, mainly adjacent to the walls. Later in the investigation (30 min), single tracks also were visible in the lumen of the alveoli and on the surface of the alveolar septa (Fig. 3b). No tracks could be seen after 60 min in the lumen of the alveoli.

In sections cut from organs of control mice, radioactive isotope injected intranasally in the same volume without virus was detected only in very small amounts, which bore no relationship to the time of the investigation.

Epithelial cells of the trachea and large bronchi of man and animals susceptible to influenza virus are usually rich in chemical receptors (mucopolysaccharides) and are capable of adsorbing the virus strongly. Nevertheless, under the experimental conditions used, only part of the virus was retained in the upper respiratory passages. It penetrated into the lower portions of the respiratory tract, including the alveoli.

Redistribution of the virus in the respiratory tract was observed 15 min after infection. In the lungs, P^{32}-labeled virus diffused from the conglomerates present in certain alveoli and spread almost throughout the lung. A similar redistribution of the conglomerates took place in the bronchi also. In the trachea, tracks could be seen near the walls, suggesting adsorption and the beginning of penetration of the virus into the tissues. The virus was found in the deep layers of the epithelium lining the trachea and bronchi 30 min after infection, possibly indicating its penetration into the cells. However, only single tracks, located extracellularly, could be seen in the lung tissue at this period. It is difficult to explain why the

redistributed virus had left the alveoli and did not penetrate into the alveolar cells. Possibly the physiological act of respiration and the less marked affinity of the virus for alveolar cells than for the tracheal and bronchial epithelium may play some role in this phenomenon. The action of cellular and humoral protective factors likewise cannot be ruled out.

The distribution of labeled virus particles in the cells of the epithelium lining all segments of the respiratory tract is noteworthy. In a few cells the tracks were located in or above the nuclei. It is considered that single tracks in the cytoplasm are evidence of commencing induction of virus protein. This is confirmed by the writers' observations on the place and time of synthesis of virus antigen in the respiratory organs of albino mice following intranasal injection of a preparation of the infectious RNA of influenza virus or of intact virus particles [1, 2, 6]. Histo-autoradiographic investigation of experimental influenzal infection in animals is worthy of note as a method for studying the primary localization of the virus and its progress among the cell systems of the respiratory organs at various times after infection.

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