Extrapulmonary Lesions in Influenza

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Influenza as an acute viral respiratory infection (AVRI) has recently been attracting particular attentions of clinicians, especially of physicians, microbiologists and virologists. Investigations by many pathologists have been devoted to this problem; they, however, studied mainly respiratory organs. These data have been presented in literature, including the prospective works of Kühn (1972), Spencer (1977), and one of the authors (Zinserling, A.V. 1972, 1977), therefore they will not be discussed in the present paper.

One observes practically in all cases of infectious diseases, particularly in the early stages of their development, a dissemination of the pathogenic agent beyond the limits of the primary site of inflammation. This may be accompanied by a development of secondary metastatic foci. Meanwhile, up to the recent time, it was taken for granted that in cases of viral respiratory infections changes outside the respiratory organs, only circulation disturbances and dystrophic changes took place, as a consequence of toxic influence from the respiratory organs.

This divergence of manifestation of AVRI and other infections compelled us to study in details other organs besides lungs (liver, intestine, central nervous system, etc.) in order to prove or reject the presence of generalization.

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The study included a complex of methods. Morphological methods included, besides macroscopic observations, first of all light microscopic examinations (stained with hematoxylin-eosin, azur-eosin, according to Hale, Brachet, Gram-Weigert and Seller, with PAS-reaction and in brain study, thionine according to Nissl, Miyagawa modified by Alexandrovskaya, according to Cajal modified by Kitoh and Matsushita.) Fluorescence microscopy was usually carried out on smears or frozen sections but for the brain tissue; it was done on paraffin sections prepared by the method of Sainte-Marie.

Electron microscopy was used in some cases. The results of morphological studies of the extrapulmonary organs were compared with the data obtained from the respiratory organ. All investigations were performed in parallel with the analysis of clinical data and of the results of pre- and post-mortem serological studies on the blood and cerebrospinal fluid (CSF), and in some cases, of virological study.

The studies of internal organs and the CNS of 680 children with an acute viral respiratory infection including 270 children with influenza A of several serotypes and B, practically always showed distinct changes associated with this infection. They were of two categories. They were the lesions characteristic of the disease: changes in epithelial cells, or less often in other cells, similar to those in respiratory organs (hypertrophy and hyperplasia of the cells in response to the viral invasion and replication). The others were non-specific alterations of the affected cells, circulatory disturbances and mild inflammatory changes of the tissue surrounding those cells.

These changes varied considerably from one case to another. They were strongest in infants and then in older children, though they were also observed even in adults, especially with immune system abnormalities. Their intensity and character also depended on the peculiarities of manifestations of respiratory viral infections. The most prominent cases were those which also caused distinct changes in the lung (parainfluenza, respiratory-syncitial virus and adenovirus infections). No wonder why the first descriptions of extrapulmonary lesions were done in cases of adenovirus infection: in the lymphoid tissue (Kawai 1959; Koroleva et al. 1967), intestine (Afanasjeva 1969; Zinserling and Shastina 1974), and the brain (Chou et al. 1973; Zinserling 1975).

Contrary to this, extrapulmonary structural changes in influenza, the most important AVRI, are less distinct than in the case of respiratory organs. There are several experimental works of Mims and Murphy (1973), Lacorte (1974), and Osetrov (1981), which showed the possibility of virus replication in the brain (chiefly in ependymocytes of the plexus chorioideus and of the lateral ventricles) followed by the development of structural changes. All these facts compelled us to study this problem. Since the changes caused by the influenza virus of different serotypes turned to be similar, they were analysed all together.
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THE RESULTS OF INFLUENZA STUDIES IN MAN

Extrapulmonary lesions of internal organs (material of Zinserling, A.V.)

Liver (the study was done partly with participation of Matveev, Y.V.). Histological examination revealed in almost half of children the changes of various degree. They were circulatory disturbances, edema of perisinusoidal space, disarray of hepatic cords with disturbance of lobular architecture and dystrophic and necrotic changes (Fig. 1a). The foci of disarray and necrobiosis in cases of more severe lesions involved central and intermediate parts and sometimes the whole lobule. The hepatocytes lost their inherent basophilic quality and were intensively stained with acid dyes in the foci of disarray. The cytoplasm of a part of such hepatocytes was uniformly eosinophilic. It contained, in other cells, small basophilic vacuoles or was disintegrated into eosinophilic lumps. Alongside with this, part of the hepatocytes and stellate endothelial cells converted into Councilman’s and Mallory’s bodies. Hyperchromic binuclear and in some cases multinuclear regenerating cells were found in rather a large quantity along the periphery of necrotic and necrotic seats. Expanded perisinusoidal space contained a homogenous liquid, floccular protein masses, granular fragments of disintegrated hepatocyte cytoplasm, and sometimes separate acidophilic bodies. Loose or more compact lympho-histiocytic infiltration with an admixture of eosinophilic leukocytes and cells resembling epithelioid ones.

Structural changes specific for influenza were rarely seen, though the viral antigen was detected in hepatocytes and stellate endothelial cells.

Macroscopically the liver as a rule was of a normal appearance. Only occasionally it somewhat increased in size and was a bit flabby.

In some severe cases clinical and laboratory data were indicative of infectious hepatitis.

Intestine (the study was performed in collaboration with Shastina, G.V.). Histological examination of 3/4 of children revealed changes mainly in the intestine (Fig. 1e, f, g). During the earlier stages of AVRI an increase in mitosis was observed in the epithelial cells. Later on quite often took place piknosis of nuclei and then peculiar rhexis with disintegration of the nucleus into small splinters of rounded or irregular form. Similar changes took place in the cytoplasm. It should be pointed out that in different sections of the intestine, even in neighboring epithelial cells, the extent of these changes might vary. In separate epithelial cells it was possible to observe rather large fuchsinophilic inclusions (FI). As this process progresses, an exfoliation of the epithelium took place. In such cases the intestinal content might consist chiefly of such exfoliating cells and fine-grain necrotic masses. In the stroma of the mucous membrane and in submucous membrane there were moderate edema, uneven blood-filling and sometimes stasis in the small vessels. Here one could observe sometimes a certain increase of histiocytes, lymphocytes and neutrophils. In the lymphatic follicles, Peyer’s patches and mesenteric lymphnodes were observed hyperplasia of the reticular cells.
Fig. 1. Changes of internal organs of children in influenza.

a: Degenerative changes of hepatocytes and stellate endothelial cells with the formation of Councilman's bodies in a child of 6 month. Azure-eosin. ×600.

b: Dystrophic changes of the tubular epithelium cells of the kidney with marked enlargement of some cells in a child of 4 month. Hematoxyline-eosin. ×1,350.
and fine-grain disintegration of some of the reticular and lymphoid cells.

In the cases of children who died between 7 and 15 days after the onset of AVRI accompanied by the symptoms of "parenteral dyspepsy" the pathological change in the intestines was of the later changes of the disease process. One may clearly see the regeneration of the epithelium.

Intestinal changes specific for the AVRI were seen in less than a half of the children. In influenza there was a considerable increase in the volume of epithelial cells being stained paler than usual. Macroscopically the intestinal changes were mild. It was insignificant dilution of intestinal content with water-like character. One can observe also a mild hyperemia of the mucosa and rarely foci of petechial bleeding.

The possibilities that these changes were caused by some other effects or were postmortem changes were denied by us after a comparative study of the same organ of children in fatal cases of other infectious and non-infectious diseases and also after comparing the severity of lesions with the time of autopsy, treatment, etc. Clinical data were also in favor of such interpretation, since the children with the most severe intestinal changes were recorded to have diarrhea during their life.

**Kidneys** (the study was performed in collaboration with Valkovitch, E.I.). Certain changes were found by microscopic examination in 3/4 of the children. The maximum lesions arose in the distal parts of the tubules (Fig. 1b, c, d). The injured cells were shed off into the lumen of the tubules in which, besides, there was protein rich fluid and sometimes erythrocytes, single neutrophilic leukocytes, and hyaline and granular cylinders. When the duration of the disease was not less than several days one observed regeneration of the tubular epithelium.

One observes also a certain increase in size of some of the glomeruli, the loops of which seemed to be swollen. The basal membrane of such glomeruli somewhat thickened, and the mesangial foundation expanded at the expense of acid and neutral mucopolysaccharide accumulation and focal stromal cell proliferation. Not seldom there was an accumulation of serous fluid in the glomerular capsules with an admixture of separate cells, basically erythrocytes and epithelial cells. The connective tissue of kidney was usually edematous, principally in the medullary layer.

Specific changes were seen mainly in nephrotherium. In influenza individual

c: Virus antigen in the epithelial cell of convoluted tubule of the kidney in a child of 6 months. Preparation treated with fluorescent serum. \( \times 1,350 \).
d: Fuchsinophilic inclusions in epithelial cells in scrape from the kidney in a child of 3 months. Methylene blue-basic fuchsin after Seller (smear). \( \times 600 \).
e: Markedly pronounced fine grain disintegration of intestinal crypt epithelium in a child of 7 months in combined AVRI, including influenza. Hematoxyline-eosin. \( \times 300 \).
f: Round cell infiltration of the stroma and regeneration of epithelium in the same child. Hematoxyline-eosin. \( \times 135 \).
g: Severe dystrophic epithelial changes in the intestine in a child of 3 months. Azure-eosin. \( \times 600 \).
Fig. 2. Brain changes in influenza.

a: Meningocytes of pia mater in a child of 2 1/2 years are increased in quantity and size. Hematoxyline-eosin. ×300.

b: Influenza virus antigen (AO) in the ependymocytes of the plexus chorioideus in a child of 9 months. Treatment with specific fluorescent serum. ×1,350.
epithelial cell considerably increased in size as a result of swelling. In such cells virus antigens and FI were demonstrated.

Macroscopically the kidneys had not undergone any changes.

Described lesions if sufficiently expressed was accompanied by distinct changes of the urine.

The study of other internal organs (cardiovascular and lymphatic systems, pancreas, adrenal glands, etc.) was performed together with other collaborators. It showed changes similar to those described above. The nonspecific processes typical for the organ in every infection disturbances of blood circulation and infectious allergic lesions were more expressed. Alongside with that, though more seldom, specific changes caused by respiratory viruses were seen.

Brain (material of Zinserling, V.A.)

Histological investigation of the brain of the 1/4 of children died with the symptom of neurotoxicosis or other neurological symptoms showed structural changes of the brain (Fig. 2). With duration of the disease more than 3 days was revealed regularly edema of the pia mater, accompanied rarely by mild lymphohistiocytic infiltration, dystrophic and ischemic changes of nerve cells. Regularly was seen the proliferation of endothelial cells of small blood vessels, followed by the thickening of their walls and an increase of their permeability sometimes accompanied by perivascular hemorrhage.

Immunofluorescence microscopic investigation showed besides these nonspecific changes respiratory virus antigens in the brain cells. When clinical symptoms lasted longer it was possible to find other lesions which could be regarded as the result of direct virus invasion.

In particular, in influenza were recorded enlargement and distinct basophilia of meningocytes cytoplasm. Such cells were grouping sometimes around injured vessels. Similar changes, though more seldom, were revealed in ependymocytes.

The brain and spinal cord were only slightly changed macroscopically; one could observe only a moderate congestion, chiefly of pia mater and signs of brain edema.

Of particular interest was a fact revealed in the study of brain lesions carried out in collaboration with Mashnski, V.F. and Aksenov, O.A. It was found that they etiologically not always coincided with the process revealed in respiratory organs at death. Thus, 15 children, including 9 infants with influenza, had doubtless brain changes due to respiratory viruses. Besides structural changes typical for this infection, the virus antigen was found in smears from leptomeninx parallel with high antibody titer in cerebrospinal fluid (CSF), while antibody titer

\[c:\] Ependymocytes from a child of 11 months, suffering from influenza B, containing viral particles in the cytoplasm. Electronogramm. \(\times 60,000\).

\[d:\] Marked sclerosis of the plexus chorioideus in a child of 3 years and 9 months suffering from influenza B and adenoviral infection. Hematoxyline-picrofuchsin. \(\times 600\).

\[e:\] Bundles of collagen protofibrils in the plexus of a child of 11 months. Also are seen singular viral particles. Electronogramm. \(\times 60,000\).
in blood was low or zero. Morphologically, there were no other symptoms of this infection except in the brain. In 3 cases sclerosis of the plexus chorioideus was found.

Such isolated brain lesions could be designated as chronic subclinical neuroinfections caused by respiratory viruses. These phenomena can be explained from the point of view of modern understanding of peculiarities of virus infection course in the brain. One may suggest that in a child with respiratory disease with viremia a temporary break of the blood-brain barrier occurs some months before the death. Later the lesions of respiratory organs ended positively. A specific infectious process occurred in the brain because of a relative autonomy of its immune system, absence of free macrophages, high specialization of cells and “tight-packing of cell component” (Johnson et al. 1978). Besides, as it is known in cases of many other viruses, influenza virus could possibly have penetrated into the brain from mucosa through olfactory nerve route.

Extremely important is the fact described by Zinserling (1977) that the lesions due to respiratory viruses, and, in particular, influenza A virus, regularly prepare a base for severe bacterial infections of the same localization, including meningococcemia.

*The lesions of placenta and fetus organs*

*Placenta* (material of Melnikova, V.F.). Quite often the placenta changed when a woman had AVRI (in particular influenza) in the last period of pregnancy. Such lesions were assisted by the immunological reconstruction of the organism necessary for the suppression of the graft vs. host reaction for fetus carrying. Blood circulatory disturbances with hemorrhages of different localization, focal acidophilic necrosis of basal membrane cells, small round cell infiltration with leukocytes inclusions were revealed in the placenta. Besides, quite often there were symptoms of premature aging manifested by massive fibrinoid accumulation, calcinosis foci, fibrosis of the stroma and vessel wall of big villi.

Specific changes were revealed in part of epithelial cells of the amniotic coverage and decidual cells of the basal membrane. These cells contained virus antigen, FT and typical structural changes. In particular, in influenza there was an increase of affected cell size and dystrophic changes.

*Fetus and newborns died within two days of life* (material of Zinserling, A.V.). This group of patients was studied to find the character of changes in respiratory viruses developing in inoculation via placenta. It is found that the lesions in such cases are similar to those described in children with air-borne infections and its later generalization. Macroscopically they were all unchanged. The only exception were the respiratory organs in which intrauterine infections caused by respiratory viruses, in particular influenza, revealed microscopically prevalence of lesions of respiratory tracts. Macroscopically the lungs were slightly infiltrated and the cut surface was covered with dark-red foci. The diagnosis of intrauterine infections was proved by the detection of virus antigen in cells and raised antibody titer in blood.
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A comparison was made (in collaboration with Vydumkina, S.P.) on the results of serological investigation of the blood of 40 children and of the blood of their mothers. In this study the antibody titer of most children was 4 times and more higher than that of their mothers. As to influenza type A1, A2, and B the titer was higher in 15, 6 and 3 patients accordingly. In the special study immunoglobulins were proved to belong to M type.

The data provided the evidence not only of the possibility of fetus infection with respiratory viruses, in particular with influenza virus, but also of severer pathology of this intrauterine infections contrary to what had been considered.

RESULTS OF EXPERIMENTAL INFLUENZA INVESTIGATION

For more accurate definition of the character of the extrapulmonary lesions we made a study on white common mice strictly of the same age (4–6 or 8–10 days).

At the first step 180 animals were intranasally inoculated with a dose of 50 LD/50 per mouse. Observations of the experimental animals included calculation of cumulative mortality rate, measurement of body weight and the size of hair development in comparison with controls. Material was taken for investigation on 3, 4, 5 and 6 days after inoculation.

Light microscopic assay was carried out by the same method as in case of autopsy materials. A comparison was made with virus content in respiratory organs, spleen and kidneys. The organs for virological tests were taken after perfusion with a large volume of physiological saline. Besides virological tests, the presence of virus antigens in these organs was determined by means of immunofluorescence microscopy according to Coons’ direct method. Besides, the levels of interferon were tested on serum, lungs and some other organs.

Mice inoculated with A virus strain (Leningrad 9/46/HO N1), which had undergone more than 40 passages in hens eggs, showed no significant difference in the development from controls. Morphological study revealed prevalent exudative reaction in the lungs, which had the character of a limited bronchial pneumonia with exudate of serum, macrophages and leukocytes, including shed alveolar epithelial cells. Generalization of the infection in animals was not recorded.

Virological tests of infected respiratory organs revealed a significant titer of virus (to 5.71 lg EID_{50}), although it was not isolated from the liver, spleen and kidneys. Concentration of interferon in mice lungs was 80–160 UN/ml during the first 2 days after inoculation, then decreased and disappeared by the moment of death (5–6 day) in the most of animals. Interferon was found in lungs and spleen during the same period (40–80 UN/ml; in kidneys (20–40 UN/ml), but it was absent in the liver during the entire course of the study.

The results differed when adapted virus A strain (Hong Kong 68/H2N2) was used. Body weight of the infected mice was about a half the control at the moment of sacrifice (2±0.4 g in contrast to 5±1 g); hair development retardation was also recorded.

The study of internal organs revealed wide and severe lesions. In respiratory
Fig. 3. Changes of internal organs of the newborn mice on the 5 day after influenza virus inoculation. Hematoxyline-eosin.

a: Dystrophic and necrotic changes of hepatocytes and disarray of hepatic cords, predominantly in lobular center. ×300.

b: The detail of the same picture as Fig. 3a. Degenerative changes of hepatocytes with formation of Councilman's bodies. ×1,300.
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organs, lesions specific for influenza were also recorded (bronchiolar and alveolar epithelium changes). The epithelial cells increased in size and their cytoplasm became acidophilic. Furthermore, they were shedding and the pneumonia was of desquamative character. Protein masses with erythrocytes, leukocytes, and macrophages accumulated in alveolar lumen. In regional bronchial lymphnodes was recorded the hyperplasia together with the rhexis of some of lymphocytes. Fi were regularly seen in epithelial cells. Generalization of infection was constant (Fig. 3). Widely spread lesions were found in the liver. Disarray of hepatic cords was noted and sharply demarcated lesions were found in the center of lobi. Vacuolization and fatty degeneration prevailed. Often were recorded the parts of liquefaction and coagulation necrosis, sometimes with Mallory’s and Councilman’s bodies. In some parts of peripheral lobi was found an increase of binuclear cells. Along with this was noted hyperplasia and degenerative changes of reticuloendothelial cells. Besides that there was edema of perisinusoidal space and an increase of lymphocytes and histiocytes content in connective tissue of the organ. Influenza virus antigen and Fi were found in hepatocytes and reticuloendothelial cells.

In the intestine an increase of mitosis was observed in the epithelial cells. Pyknosis of the nucleus took place and then rhexis with disintegration into small splinters of round form. In the stroma of mucous membrane there was a moderate edema. In some of the reticular and lymphoid cells were observed hyperplasia and fine-grain disintegration.

The kidneys changed less markedly. Nephrothelial cells in tubules increased their volume and showed signs of vacuolar dystrophy. The nuclei of these cells were stained faintly or could not be determined. Protein masses were present between the tubules. Endothelial cells of some arterioles and some glomerular capillaries were enlarged and edematous. In some glomeruli were defined PAS-positive masses. Immunofluorescence study revealed virus antigen in nephrothelial cells and Fi were found by light microscopy.

In the brain was recorded a moderate congestion of pia mater together with mild lymphohistiocytic infiltration and also dystrophic changes of nerve cells. Disturbances of endothelial cells and small blood vessels were regularly registered, including plexus chorioideus. Viral antigen was found in injured cells.

The spleen has undergone severe changes. Necrosis of its tissue up to 1/3 was observed mainly in follicle centers. In these parts the cells underwent rhexis into small splinters and acidophilic bodies were found there. When cell rhexis was eliminated some of reticular cells were enlarged in size. Peripheral sinuses were expanded and they contained the desquamated lining cells, reticular cells, serous

c: A large quantity of mitosis in the intestinal epithelium and fine grain disintegration of single cells. ×600.
d: Dystrophic changes and proliferation of tubular epithelial cells in the kidney. ×1,350.
e: Increases of pia mater meningoeytes in size and quantity. ×600.
f: Rhexis of lymphocytes in lymph follicles of the spleen. ×300.
fluid, separate erythrocytes and small splinter masses. Virus antigen and FI were found in the cytoplasm of reticular cells.

In some parts of myocardium were noted dystrophy of muscle cells and histiocytic infiltration in stroma.

Virological study revealed a trustworthy increase of influenza virus content in the organ (Fig. 4). Maximal virus content in the lungs was recorded on the 3 day (5.71 EID<sub>50</sub>), its concentration decreased before death to the level of 3.5–4.0 lg. Viremia was recorded on the 2–3 days. Virus content in the liver, spleen, kidneys was on 1.5–2.0 lg more than in the blood.

Interferon was isolated only from lungs in small quantities (20–40 UN/ml during first two days, then it decreased and became undetectable). There was no interferon in the liver, spleen or kidneys.

Thus, using influenza A strain adapted to mice, inoculation of newborn mice provokes severe lesions of respiratory organs with further development of extrapulmonary lesions. They are caused mainly by the virus replication in these organs. One of the main reservoirs of the extrapulmonary virus is the liver. One of the reasons of such massive accumulation of adapted virus strain in the organs may be explained with its larger resistance in comparison to unadapted virus strain to interferon and other immunological factors of the defence.

The second step of the investigation was devoted to the special study of some extrapulmonary seats of generalization, particularly in the brain. The trial was made on 110 white common mice of the same age. We managed to specify the character of these lesions and determined that in case of nasal inoculation of mice
with meningococci they could superpose to viral infection of the central nervous system.

**DISCUSSION**

Presented data prove the possibility of influenza and other AVRI generalization with development of extrapulmonary lesions. They are characterized by the same kind of the changes at the cellular level regardless of their etiology and localization, and consist of mainly degeneration, proliferation and in lesser degree exudation.

More regularly such lesions take place in children of the younger age; they were reproduced experimentally by aerosol inoculation to the newborn mice. The factors promoting the dissemination of the agent and development of generalized infection include the virulence of the agent, and the state of the host, i.e. immaturity of internal organs, especially of the immune system. Such kinds of lesions in the respiratory virus infections bring them together to other viral infections caused by herpes virus, cytomegalovirus, enteroviruses, the generalization of which is well known under the definite conditions.

Our investigation showed that for accurate diagnosis it is necessary to use both purposeful histological and immunofluorescence study of the organ together with serological study, and in case of brain lesion in comparison with cerebrospinal fluid study, if possible in dynamics.

The questions studied in the present work do not enclose the whole problem but show its doubtless importance for the medical theory and practice. The collaborative research of the scientists of different countries is required for the successful resolution of this problem.

**References**


