

## CHARACTERISTICS OF INFLUENZA VIRUS STRAINS, ISOLATED IN 1963 IN VARNA

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In March 1963 7 strains of influenza virus type A<sub>2</sub> were isolated at the virological laboratory of the Higher Medical Institute — Varna from local inhabitants, suffering from influenza. Their etiologic significance in the disorders, observed in this period, was proved by examination of the haemagglutination inhibition test (H.I.T.) by means of paired samples of blood sera, obtained from sick individuals as well as from those who have already suffered from the disease. In some of the examined individuals a 4 and more fold rise in antibody titre was established (5).

We set ourselves the task to investigate the antigenic structure of the isolated influenza virus strains.

### Materials and method

Washings of the naso-pharyngeal cavity of the patients were examined. These were obtained in the first one-two days following the onset of the disease. The materials were processed with chicken erythrocytes for an hour at +4°C immediately after they were obtained. Centrifugation at the same temperature followed.

Side microflora was removed by treating the erythrocyte suspension with penicillin, streptomycin and nystatin. The materials thus processed were examined on 10—11 day chicken embryos by means of combined contamination in the amniotic and allantoic cavity with 0.1 ml erythrocyte suspension each. Cultivation of the inoculated chicken embryos was carried out at 32—35°C. The method was based on the results obtained in the observation (2, 6) in 1957 and 1959, as well as in those performed in Varna in 1962 (3, 4). Virus contents in the amniotic and allantoic fluid of the embryos was established by means of haemagglutination inhibition test (H.I.T) with 1% suspension of chicken erythrocytes.

The antigenic properties of the isolated strains were studied by means of HIT with 1% of chicken or human erythrocytes belonging to blood group 0. As standard influenza diagnostic sera (produce of the Scientific Research Institute for Epidemiology and Microbiology) served the allantoic fluid of the strains A Shkljaver: A<sub>1</sub> — Ned, A<sub>2</sub> — Sofia 1/57, A<sub>2</sub> Sofia — 1960, A<sub>2</sub> Angl., B — Lee and D — Send. obtained in two AU. The studied 7 strains were continuously used as a fresh virus containing allantoic fluid.

As standard influenza diagnostic sera were used the specific anti-influenza sera, obtained from cocks, against the vira A-Shkljaver, A<sub>1</sub>-Ned, A<sub>2</sub>-Sofia 1/57, A<sub>2</sub>-Sofia-1960, B-Lee, D-Send and C. Sera specific against the 7 isolated strains were obtained by means of immunization of albino rats. The latter were contaminated at the beginning by intranasal route with 1 ml and intraperitoneally with 2 ml of fresh allantoic fluid containing high titre of influenza virus. The second injection was administered intraperitoneally two weeks later with 2 ml of fresh virus containing allantoic fluid.

Two control groups of rats were used in the experiments: the rats from the first group were inoculated with normal allantoic fluid, whereas the second group of rats remained under the same environmental conditions without being treated whatever. Total blood letting of the rats was performed on the 12 day following the last inoculation. All specific sera obtained, as well as the standard ones, were processed prior to the experiment with CO<sub>2</sub> and were inactivated at 58°C for 30 minutes.

The inhibitory sensitivity of the strains was determined by means of horse and guinea pig sera, whereas their haemagglutination activity was measured by using mice and bovine erythrocytes.

To determine the infectious titre chicken embryos were used intrallantoically contaminated by means of a renfold dilution of the virus. Embryos were cultivated at +34°C.

The toxicity of the strains was determined after A. Ya. Zakastelskaya's method (1) by means of intravenous injecting of albino mice and in the anterior ocular chamber of rats.

## Results

The influenza virus, which caused the disorders in 1963 in Varna, does not differ much after its adaptational properties for cultivation on chicken embryos from type A<sub>2</sub>, which caused the epidemics in 1957 and 1959 in the country and in 1962 in Varna. Only 1—2 passages proved sufficient to reach a virus contents of 1 : 20—1 : 80 in the allantoic fluid of the inoculated chicken embryos, manyfoldedly increasing in subsequent passages.

A detailed serologic study by means of a cross haemagglutination inhibition test (Fig. 1) revealed some peculiarities of the isolated strains. All seven strains used for immunization of rats, stimulated the production of antibodies, some of them with a significantly high titre (1 : 5120) with regard to the homologous antigen. The isolated strains, strain 24 being excluded, displayed a comparatively slight reaction with the heterologous standard sera. Serum A<sub>2</sub>—Sofia suppresses haemagglutination of all isolated strains at least to half the initial titre. In strain No. 24 this was observed twice. The influenza diagnostic serum A<sub>2</sub>—Sofia also reacts with the specific antiinfluenza rat sera, obtained against the isolated strains. At the same time these sera do not react in HIT with the other standard influenza diagnostic sera, with the exception of A<sub>2</sub>—Sofia.

This definitely indicates that all the isolated strains of influenza virus belong to type A<sub>2</sub>. The positive serologic findings, witnessing the role of type B in this epidemics (5) were not virologically confirmed by us.

The antigenic relation between the isolated influenza strains is confirmed by the positive results of the cross HIT reactions in which only rarely inhibition of haemagglutination is observed, lower than half the titre of the serum and in some cases the coefficient of surpassing the

Sera		Standart Sera					Sera against isolated Influenza Strains							Titre of homologous sera			
		A	A <sub>1</sub> Ned	A <sub>2</sub> Sofia	B Lee	B Sofia	D	1	3	6	15	24	33		1234		
Standard Strains	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	640	
	A <sub>1</sub> Ned	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2560	
	A <sub>2</sub> Sofia	—	—	—	—	—	—	2	—	—	—	—	—	—	—	1280	
	B Lee	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2560	
	B Sofia	—	—	—	—	—	—	—	—	—	—	—	—	—	—	640	
	D	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1280	
	Influenza Strains recovered in the year 1963	1	—	—	—	—	—	—	—	—	—	4	—	2	2	—	2560
		3	—	—	—	—	—	—	—	—	—	—	2	—	—	2	5120
		6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	160
		15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	640
		24	—	—	2	—	—	—	—	—	—	—	2	4	—	—	1280
		33	—	—	—	—	—	—	—	—	—	—	—	4	—	—	2560
		1234	—	—	—	—	—	—	—	—	—	—	—	—	2	—	1280

Fig. 1

homologous titre reaching even 4 (Vid. Fig. 1). All this reveals a comparatively monolythis antigenic structure of the isolated strains. Nevertheless it is quite difficult to solve the problem of the avidity of the isolated strains based on these data only. Of the latter inhibitory sensitive to horse and guinea pig sera are the strains 1, 15, 24 and 23, which at the same time do not agglutinate mice erythrocytes. On the contrary, strains 6 and 1234 are inhibitory insensitive and result in haemagglutination with mice erythrocytes of 1:16 — 1:32.

All freshly isolated strains appeared atoxic to albino mice and rabbits. Their infectious titre does not differ from that of strains isolated in the epidemics of 1962 (4).

The obtained data gives us ground to draw a conclusion that the seven influenza virus strains type A<sub>2</sub> isolated in 1963 do not differ substantially from the influenza strains A<sub>2</sub>, isolated in 1962 in Varna. The difference between them consisted in the fact that the strains isolated in 1962 displayed a more marked avidity to heterologous sera A, A<sub>1</sub>, B, D, C as compared with those isolated in 1963. More detailed studies of the

isolated strains comparing their antigenic structure with the strains A<sub>2</sub>-Angl., A<sub>2</sub>-Sofia 1/57, A<sub>2</sub>-Sofia-1960 indicate a more close relation with the latter two strains. This fact confirms the view that type A<sub>2</sub> continues to change also at present its antigenic structure. This imposes a continuous follow-up of influenza morbidity, also studies of the antigenic structure of the influenza virus causing epidemics in various countries.

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### ХАРАКТЕРИСТИКА ШТАММОВ ВИРУСА ГРИППА, ВЫДЕЛЕННЫХ В ГОРОДЕ ВАРНЕ В 1963 ГОДУ

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#### РЕЗЮМЕ

Авторы проводят систематические наблюдения над заболеваемостью гриппом в городе Варне. В марте 1963 г. были выделены 7 штаммов вируса гриппа типа А<sub>2</sub>. На основании проведенных исследований антигенной структуры выделенных штаммов было установлено, что 4 из них являются авидными и 2 неавидными вариантами типа А<sub>2</sub>.

Выделанные штаммы вируса гриппа в 1963 г. по своим свойствам не обладают существенными отличиями от штаммов, выделенных в городе Варне в 1962 г.