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**A COMPARATIVE STUDY  
OF THE  
INTRANASAL AND AEROSOL  
METHODS OF VACCINATION  
AGAINST INFLUENZA**

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**U.S. ARMY BIOLOGICAL LABORATORIES  
FORT DETRICK, FREDERICK, MARYLAND**

**A COMPARATIVE STUDY OF THE INTRANASAL AND AEROSOL  
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**U.S. ARMY BIOLOGICAL LABORATORIES  
FORT DETRICK, FREDERICK, MARYLAND**

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A Comparative Study of the Intranasal and Aerosol Methods of Vaccination Against Influenza

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Ivanovskiy Institute of Virology AMN USSR.

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An active specific prophylaxis of influenza is the most important measure that can be carried out in the struggle with this infection. The mass spreading of influenza both during epidemics as well as during inter-epidemic periods gives rise to the necessity of developing methods of application of the influenza vaccine that would be the least labor consuming and ensure the greatest mass character of immunization.

In recent years Aleksandrov and Gefen have suggested a method of aerosol vaccination against a number of infectious diseases which is intended for emergency and mass immunization of a large number of people. Isolated attempts at utilizing the aerosol method of immunization against influenza were carried out even earlier (Smorodintsev with co-authors), however during the inhalation administration of an aerosol of liquid live influenza vaccine, high reactogenicities turned up in the investigations of these authors. The method of aerosol immunization developed by Aleksandrov and Gefen differs from those applied earlier with the use of a polydisperse fractional composition with particle sizes from 1 up to 200 microns.

The aim of the present work is a comparative study of the intranasal and aerosol methods of vaccination against influenza, and also the reactogenicity and immunological effectiveness of live influenza vaccines of different construction.

For the investigation, three types of type A live influenza vaccine were prepared at the Mechnikov Institute of Vaccines and Sera in Moscow in February of 1961. These were egg tissue, egg, and egg tissue combined. The vaccine included strains which were used in making vaccine in 1960, and also vaccinal strains selected and recommended by the laboratory for vaccine prophylaxis of influenza at the Institute of Virology of the AMN, USSR.

The vaccine was prepared in accordance with the industrial instructions in force.

For the intranasal immunization the vaccine was subjected to lyophilization and issued in ampules with 20 doses in each. The vaccine was sterile and harmless, and its biological titer equaled  $10^{-6}$  -  $10^{-7}$ .

For the aerosol immunization out of the same series of vaccine, a dry pulverized influenza vaccine was prepared with a biological titer equal to  $10^{-5}$  -  $10^{-6}$ . The vaccine didn't contain pathogenic microflora.

The biological and antigenic activity of the designated series of vaccines was studied in virusological and serological tests at the Institute of Virology of the AMN, USSR. For this end, they diluted the vaccine from  $10^{-2}$  to  $10^{-8}$ , and then with each dilution of the virus they infected 4 chick embryos which were in the 11th day of incubation. The egg variant of  $A_2$  vaccine had a biological titer of  $10^{-7}$ , after lyophilization the titer was preserved. The biological titer of the egg variant of the vaccine for aerosol application was lowered by 2 logarithms after lyophilization - from  $10^{-7}$  to  $10^{-9}$ . The biological titer of egg tissue  $A_2$  vaccine, prepared from  $A_2$  strain (Rim, 1960) for intranasal use, was lowered by one logarithm - from  $10^{-7}$  to  $10^{-8}$ . Egg tissue combined  $A_2$  vaccine, prepared for intranasal and aerosol application, preserved the initial titer of  $10^{-7}$  after lyophilization.

We took under observation 512 men who were 18-21 years old and were under similar conditions of work and daily living. In March 1961 an immunization was conducted with various types of live influenza vaccines by the intranasal and aerosol methods. Personnel of the control groups received a preparation, containing all the ingredients which form a part of vaccines other than the vaccinal virus of influenza ("placebo").

The intranasal vaccination was carried out in accordance with the directions for the use of live influenza vaccine. Before use they diluted it in distilled water and administered it with the help of liquid pharmaceutical sprays in a dose equal to 0.1 mg, that is 0.5 ml of diluted vaccine.

The aerosol immunization was carried out in a room with a volume of 50 m<sup>3</sup>, in which 35-40 men were vaccinated simultaneously. The vaccine was dispersed with the help of glass atomizers during a 15 minute exposure. The doses of vaccine inhaled during the aerosol immunization constituted 0.05, 0.09 and 0.15 of the intranasal dose.

In as much as it is not possible here to carry out a complete conclusion of the equation for determining the average weight concentration of vaccine aerosol, we will limit ourselves to just a brief analysis of it:

$$G_v = 0.015 \left[ \sum_1^m \frac{1}{2} \frac{G_n \cdot G_0}{W} + \sum_{n+1}^n \left( \frac{G_n \cdot G_0 \cdot T_n}{W \cdot T_0} - \frac{1}{2} \cdot \frac{G_n \cdot G_0}{W} \cdot \frac{T_n^2}{T_0^2} \right) \right]. \quad (1)$$

The following meanings are used in the equation: n-overall amount of aerosol fractions; m-number of fractions, the sedimentation time of which is greater than the duration of the immunization sitting;  $G_v$ -average weight concentration of vaccine aerosol;  $G_n$ -weight percentage of the corresponding fraction;  $T_n$ -sedimentation time of this fraction;  $G_0$ -pulverized weighed portion of vaccine; W-volume of the room in which the immunization is carried out;  $T_0$ -duration of the vaccination seance.

They determined the inhaled dose from the equation:

$$AD_{ves} = G_v \cdot T_0 \cdot V_{lv}, \quad (2)$$

where  $AD_{ves}$ -inhaled dose of vaccine by weight;  $V_{lv}$ -volume of pulmonary ventilation.

The fractional-dispersible composition of the influenza vaccine was as follows: from 1 to 10 microns - 0.4%, 11 to 25 microns - 12.5%, 26 to 50 microns - 21.9%, 21\* to 100 microns - 33.2% and from 100 to 200 microns - 32%.

The volume of the room in which the vaccination was conducted was 50m<sup>3</sup>. In the first experiment, the suspension of pulverized vaccine constituted 6g, in the second - 12g, and in the third - 20g; 1 gram of dry aerosol vaccine contained 33 intranasal doses (under conditions where the rules of preparation were acceptable to us).

Based on these facts it is easy to determine the inhalation doses, both by weight and in conversion to intranasal doses (table 1).

Table 1.

No. of Experiment	Suspension of Vaccine G <sub>0</sub> (in g)	Volume of room W (in m <sup>3</sup> )	Inhalation Dose	
			Weight (in mg)	In intra-nasal doses
1	6	50	1.38	0.05
2	12	50	2.76	0.09
3	20	50	4.6	0.15

With the aim of clearing up the effect of pulverization on the ability of the virus to survive, a collection of air samples was carried out with the help of special devices and with a subsequent determination of the live virus concentration in them. The vaccinal virus was detected in considerable quantities in all the investigated air probes.

The reactogenicity of the vaccines used in the aerosol and intranasal methods was determined by studying the general and local symptoms of vaccinal reaction at 48, 72 and 96 hours after immunization or administration of "placebo." With this aim, from the second day after the vaccination and running for three days, before the vaccinated persons went to sleep their body temperature was measured. Complaints were recorded of dysphoria, headaches, tickling in the throat, and the presence of head colds and sneezing.

In a comparison of reactogenicity, based on a temperature reaction caused by an optimum dose of vaccine (0.09 intranasal dose when administered by the aerosol method and 0.1 mg by the intranasal method), it is noted that during intranasal administration there were 21.3% of temperature reactions, out of which 2.7% are higher than 37.5°, and during aerosol administration there was a 9.2% temperature reaction and none of these were higher than 37.5°.

In the control groups a temperature reaction was noted; during intranasal administration, in 5 men out of 35 (14.2%), and during aerosol immunization in 2 out of 39 (5%). On this basis it follows to suggest that temperature reactions due to the vaccinal virus consisted of 7% during the intranasal method and 4.2% in the aerosol method. Complaints of headache were noted in 42 men out of 108 (38.8%) during the intranasal method of immunization, and in 39 men out of 130 (30%) during the aerosol method. During the intranasal method the emergence of head cold was noted in 47 men out of 108 (43%), and during aerosol - in 33 out of 130 (25.4%).

During the aerosol method of immunization, less symptoms of vaccinal reaction were observed than during the intranasal method, except tickling in the throat which was observed more often during the aerosol method. With this it has to be noted that the symptoms during the aerosol method were observed in the course of one day and were little expressed. There wasn't one case of resorting to a doctor in connection with the vaccinal reaction.

In a number of cases, complaints and the appearance of symptoms were observed with the administration of "placebo." This is explained not by the influence of the vaccinal virus but of the reactions of the organism to the administration of the ingredients included in the composition of the vaccine.

We studied the ability of the virus to become accustomed to the mucous of the respiratory tract by taking nasopharyngeal washings from vaccinated persons and subsequently infecting chick embryos. Nasopharyngeal washings were collected from all the vaccinated persons after 48, 72 and 96 hours and after the appropriate processing of the virus containing washings, they carried out the infection of 4 chick embryos. After 72 hours of incubation at 35°, the chick embryos were examined and the presence of the virus was determined in them.

The ability of A<sub>2</sub> virus to become accustomed to the mucous membrane of the respiratory tracts during the intranasal application was noted in 78-91% of those vaccinated. During the aerosol method of applying the vaccine in a dose of 0.05, the virus took root in 20.6-51.1%. With an increase in the amount of vaccine from 0.09 to 0.15 intranasal dose, there was noted a high acclimatization of the virus - in 60.8-85.3% of those vaccinated (table 2).

Besides studying the ability of the vaccine to take root in the mucous of respiratory tracts, they also studied the immunological progress in those vaccinated. For this end they took blood from each vaccinated person prior to the vaccination and then after 3 weeks following the vaccination. The paired sera were studied on the dynamics of the increase of the titer of antibodies in the reaction of retarding hemagglutination. They set up the reaction with antigens of the virus included in the composition of the vaccine. During the intranasal method, the increase of the titer of antibodies is noted in 57.6-73.3% of the sera. The aerosol administration of an 0.05 intranasal dose caused an increase in the titer of antibodies in 55-83%, and with an administration of the 0.09 and 0.15 intranasal dose - in 73-94% of the sera.

#### CONCLUSIONS

1. The reactogenicity of influenza vaccines during the aerosol method of immunization was lower than during the intranasal method. Along with this there were no temperature reactions over 37.5° noted. Other symptoms of vaccinal reaction (head cold, headache, sneezing and others) were observed less seldom during the aerosol method and weren't so sharply expressed.
2. The egg tissue and egg-tissue combined type A<sub>2</sub> vaccine provided the best indices of adaptability and immunological effectiveness.
3. Comparative data testifies to the future prospects and convenience of the aerosol method for the immunization of large masses of the population with live influenza vaccine, however for a conclusive evaluation of it, further investigations are required.



LITERATURE

Aleksandrov, N. I., Gefen, N. Ye., Military Medical Journal, 1958, No. 10, p. 62.

\*Translator's note. Probably should be 51.

Adaptability & Immunological Shifts in Persons Vaccinated by Various Methods & Variants of Vaccines

Method of Vaccination	Dose of Virus	No. Vaccinated	Type of Vaccine	No. vaccinated in which the virus took root in various terms (in hours)				Extent of response to vaccination (%)	No. of paired sera investigated	Coefficient of Increase of Antibodies					Immunological shifts present (%)
				48	72	96	2x			4x	8x	16x			
Intranasal	0.5mm of diluted vaccine	38	egg	--	26	22	78.9	33	7	2	--	60.6			
		37	egg tissue	--	26	12	81.8	30	6	1	--	73.3			
		33	egg tissue combined	--	24	28	91.4	33	5	--	--	57.6			
Aerosol	0.05 of intra-nasal dose	28	egg	4	4	--	20.6	23	6	1	--	83			
		32	egg tissue	12	4	--	45.1	27	4	3	1	70			
		29	egg tissue combined	9	9	--	51.5	29	8	5	3	--	55		
Aerosol	0.09 of intra-nasal dose	45	egg	11	16	15	60.8	39	14	7	1	84.6			
		41	egg tissue	16	27	16	76.7	37	16	7	3	94			
		44	egg tissue combined	19	25	11	85.3	40	8	6	5	75			
Aerosol	0.15 of intra-nasal dose	35	egg	12	22	7	69	30	5	2	--	71			
		39	egg tissue	9	16	10	71	33	9	4	3	78			
Aerosol		37	egg tissue combined	19	16	7	70	37	11	4	--	73			

Table 2.