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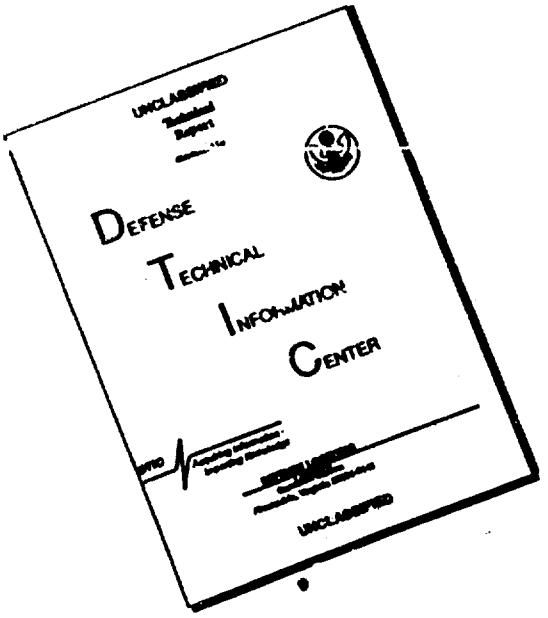

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Experiment of Mass Aerogenic Vaccination of Humans Against Anthrax*

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Certain theoretical and experimental data of our research of several years' standing on the problem of aerogenic immunization were already published in a brief form**.

Accumulated data on the studies of "reaktogennost" [reactive efficiency] and effectiveness of the cited method of immunization, obtained in experiments on animals (guinea pigs, rabbits, sheep, [illegible in original]), as well as positive results of studies of the method on people, permitted to proceed, with the permission and on the recommendation of the Committee of Vaccines and Sera of the Ministry of Public Health USSR, to a wider approbation of the method of aerogenic immunization by the dust anthrax vaccine; it was produced in March 1959.

The present work is the first experiment of mass aerogenic vaccination of people, and, therefore, its results are of decided interest. We set the following problems when conducting the work: to test and make more precise the method of mass aerogenic vaccination of people under practical conditions; to test and determine more accurately the previously obtained data about reactive efficiency of immunization against anthrax; to define the comparative effectiveness of various methods of immunization against anthrax with the aid of the specific allergen, which was developed and suggested, in 1957, by E. N. Shlyakhov under the name of "anthrax allergen MIEMG."

The aerogenic vaccination was conducted in an ordinary room (size 40 m^3) of the district hospital (area 3.7 x 3.6 m; height 2.9 m) with one window and one entrance door. Radio

* This work was conducted in the Moldavian SSR under the leadership of Under Secretary of Public Health MSSR, V. A. Malygina, with the assistance of N. N. Ezhov, M. G. Ostapenko, A. S. Goreshter and E. V. Gruz.

** See Voyenno-Meditsinskiy Zhurnal, Nos 10, 11 and 12 for 1958.

was provided in the room, and it was equipped with benches and chairs. The vaccine was diffused from two apparatuses that were installed symmetrically along the line, which divided the room lengthwise and in two; apparatuses were placed away from the walls at a distance of 1.8 m, 1.23 m and 2.46 m, and at a height over the floor equal to 2.4 m.

During each process of immunization, 40-50 people were vaccinated simultaneously in such a room. Five series (Nos 2, 13, 16, 23 and 27) of the aerogenic vaccine from strains STI-1 and No 3, with an initial activity of from 20 to 2,500 billion of spores in one gram, were utilized for the immunization.

Depending on the initial activity of the vaccine, we placed in each atomizing apparatus from two to three grams of vaccines. Consequently, in all, from four to six grams were dispersed in the room. Atomization took place continuously during the course of the entire exposure to immunization, which lasted from 5 to 15 minutes.

The settings for immunization were conducted in a similar room, but of a size of 20 m^3. In the given dose it proved to be sufficient to use one atomizing apparatus in order to create the necessary concentration of the vaccine in the air; it was placed in the center of the room. Estimation of living microbes in the air of the room, as well as determination of the aerogenic immunizing done, obtained by each of the participants in the test, was produced by the method of test samples of air intake, through a gas mask, that was equipped with a foam-gelatin filter at the inlet. Specimens were taken during the course of the entire exposure to immunization by two men at the same time (with a certain one minute volume of lung ventilation), who were present in the room together with those being vaccinated. Thus, on the filter was retained the amount of microbes, which a man could breathe in during the time of aerogenic immunization, that is, equal to the aerogenic immunizing dose. Determination of this dose was achieved by means of dissolving the filter in physiological solution, with following tenfold dilutions and sowings on D agar. Knowing the aerogenic dose of exposure, as well as the one minute volume of lung ventilation, it was possible to calculate the mean concentration of living microbes in the air of the room by means of dividing the dose in the product of the one minute volume and the exposure.

Taking into consideration that doses of the aerogenic anthrax vaccine in the limits of 50-500 min living microbes created an immunity of sufficient intensity. [Illegible] preliminary tests were borne by people without any reactions, we decided to use the same doses in the present work.

In all, 363 persons were subjected to aerogenic vaccination; of these 220 to doses which comprised tens of millions of living microbes (from 15 to 63 min), and 143 to doses, which comprised hundreds of millions of living microbes (from 440 to 640 min). All the persons, who were to be subjected to aerogenic vaccination, underwent an examination at the dispensary, which included medical examination, clinical analyses of blood and urine, as well as X-ray examination of chest organs. Essentially healthy persons of this group, in the age group 18-45 years old, who worked at the kolkhoz, were subjected to immunization.

Medical observation of the state of health of the vaccinated was established at once from the moment of immunization and was conducted in 263 persons during the course of seven days, and in 100 during the course of 21 days. Besides the daily taking of temperature, on the first, third, fourth and sixth day of observations, certain of the immunized (100 persons) were subjected to X-ray examinations of chest organs, as well as to clinical analyses of blood (the number of leucocytes and ROE [erythrocyte sedimentation reaction]).

It was established, as a result of observations that among the vaccinated not one person had either a local or general clinically expressed reaction. No temperature reaction was observed in anybody, neither any other changes in the state of health. All the vaccinated persons continued to conduct the agricultural work on the preparation for the spring sowing campaign. X-ray examinations of chest organs of vaccinated persons, conducted in one, three and six days after immunization, also did not show any changes. One should point out that in 19 persons, who underwent aerogenic vaccination, some changes in the lungs were observed during dispensary examination; they indicated that these persons underwent a tuberculosis process in the past (petrifications, sinus adhesions, pleural superpositions ["nalozhenita"], interlobular twists ["shvarty"] and others). Further observations did not show any aggravations of the existing changes or deterioration of health conditions in a single person.

A temporary change in the total amount of leukocytes of the blood and "ROE" [erythrocyte sedimentation reaction] were the only clinical symptoms of the response reaction of the organism to the inhaled vaccine.

The results of hematologic changes in persons who were immunized with the aerogenic anthrax vaccine, as compared with similar indicators before the vaccination, are cited in Table 1.

Table 1

Time of examination after vaccination	Number of the examined	Number of leukocytes after immunization: Decrease[1]	Without change	Increase by: 500-1,000 leukocytes	1,100-3,000 leukocytes	3,100-5,000 leukocytes	No of persons with an amount of leukocytes that exceed the norm[2]	"ROE" after immunization: Retardation	Without change	Acceleration	Acceleration exceeding norm[3]
1 24-hour day	28	4	2	10	5	7	8	2	9	17	6
3 24-hour day	28	5	2	7	8	6	7	5	13	10	4
6 24-hour day	19	5	4	6	4	-	2	-	-	-	-
In all for all dates	75	14	8	23	17	13	17	10	30	35	11

Footnotes: 1. Decrease in the number of leukocytes, in comparison with the given indicators before vaccination, were not below the norm in a single case (5,000 leukocytes in 1 mm^3 of blood).
2. The upper limit of the norm was assumed as 9,000 leukocytes in 1 mm^3 of blood.
3. The upper limit of the norm for men was assumed as 10 mm/hour, for women as 15 mm/hour.

It is seen from the cited data, that already in 24 hours after vaccination an increase in the amount of leukocytes was noted in most of the examined (22 out of 26). Increase of leukocytes, basically, was within the limits of the norm. Nevertheless, in eight cases it comprised 9,300 - 10,200 leukocytes in 1 mm^3 of blood. Along with this in 17 persons out of 28 was noted an accelerated "ROE" [erythrocyte sedimentation reaction]; whereupon in six -- with an insignificant exceeding of the norm (from 15 to 20 mm/hour. Similar changes took place also in three days after vaccination; there was one difference only, that the number of persons with the acceleration of "ROE" was reduced.

On the sixth day after vaccination, only half of all the examined showed an insignificant increase in the number of leukocytes; whereupon, only in two cases it exceeded the norm. At this time of examination, erythrocyte sedimentation reaction also, as a rule, became normal. Thus, the cited data ascertain that the aerogenic anthrax vaccine, which was used

in reasonable doses, proved to be virtually "areaktogennoi" [areactogenic -- producing no reaction?]. Nevertheless, distinct changes on the part of the blood, which were discovered after the immunization, indicate the presence of a general reaction of the organism to the vaccine that proceeded in a subclinical form, and which can be discovered only through laboratory methods of research.

Immunologic effectiveness of the aerogenic anthrax vaccine was studied with the aid of the specific allergen of the Moldavian "IEMG" [Institute of Epidemiology, Microbiology and Hygiene?] (series 22 and 24). The allergen was diluted with physiological solution in a ratio 1:1 before its use and was injected intracutaneously into the forearm of the left arm in the amount of 0.05 ml. For the control of the specific reaction, physiological solution was introduced into the forearm of the right arm in the same amount. The reaction was evaluated in 24 and 48 hours according to a time scale which was developed by E. N. Shlyakhov. Simultaneously with the conducted aerogenic vaccination, we studied, in the same way, the immunologic effectiveness of the vaccine STI in its subcutaneous and cutaneous applications. Vaccination was conducted in precise conformity to the existing instructions for the application of this vaccine. In all 250 persons were vaccinated subcutaneously and 250 -- cutaneously. Comparative indicators of immunological effectiveness of the anthrax vaccine in its aerogenic, subcutaneous and cutaneous application are presented in Table 2.

It is seen, from the cited data (in Table 2), that the positive allergic reaction was registered in aerogenically vaccinated people, as well as in those immunized subcutaneously and cutaneously, already on the seventh day. In the group of persons, aerogenically vaccinated, the number of positive reactions, at this time of examination, was smaller than in the group of people who were vaccinated subcutaneously. We found an explanation for this fact in the matter that among the number of aerogenically vaccinated and examined on this date were only the persons who were immunized with a dose which comprised tens of millions of microbe bodies (42-63 min), and did not prove to be the best.

Somewhat different proportions were obtained on the following dates of examination of those vaccinated by different methods, where in the groups of aerogenically vaccinated, together with persons who were immunized with a dose of tens of millions of microbe bodies, there was a considerable number of those who were vaccinated with a dose of hundreds of millions of microbe bodies (440-660 min).

Table 2

Time of examination after vaccination	Method of vaccination	Number of the examined	Number of persons with: Reactive allergic reaction	Ineffective reaction	Doubtful allergic reaction	Number of persons with a positive allergic reaction: In all	+	++	+++	++++
7th day	Aerogenic	19	7	1	2	9	9	-	-	-
	Subcutaneous	25	4	1	3	17	5	5	7	-
	Cutaneous	25	10	-	4	11	3	6	2	-
15th day	Aerogenic	26	4	-	3	19	4	7	6	2
	Subcutaneous	49	18	-	8	23	11	7	5	-
	Cutaneous	50	20	2	6	22	5	10	7	-
30th day	Aerogenic	64	21	1	11	31	14	9	8	-
	Subcutaneous	50	14	4	14	18	8	-	10	-
	Cutaneous	50	22	2	14	12	6	2	4	-
90th day	Aerogenic	52	8	1	8	35	18	9	7	1
	Subcutaneous	52	15	2	8	29	12	4	11	-
	Cutaneous	24	7	2	6	9	8	1	-	-
	Control (unvaccinated)	70	50	9	7	4	4	-	-	-

Footnote: To the ineffective were referred the reactions where hyperemia was present after the application of both the allergen and the physiological solution.

About 3/4 of the vaccinated, who were aerogenically vaccinated, reacted positively to the allergen in 15 to 90 days after vaccination. In groups of those vaccinated subcutaneously and, especially, cutaneously the number of positively reacting was smaller. Positive allergic reaction occurred in 30 days in half of all the examined in the group of aerogenically vaccinated, while in those inoculated subcutaneously and cutaneously -- approximately in 1/3.

In connection with the fact that the size of the inhaled dose of the vaccine essentially influenced the indicators

of immunological effectiveness, and the maximum doses tested by us (440-660 min. microbe bodies) were especially favorably endured by the people, the allergic reactions in persons who were immunized with these doses, are of special interest.

Results of the allergic reaction in the aerogenically vaccinated persons, depending on the immunizing dose, are shown in Table 3.

Table 3

Time of examination after vaccination	Dose of aerogenic vaccine	Number of the examined	Number of persons with			Number of persons with a positive allergic reaction				
			Negative allergic reaction	Ineffective reaction	Doubtful allergic reaction	In all	+	++	+++	++++
7th day	43-63 min. microbe bodies	19	7	1	2	9	9	-	-	-
15th day	43-63 min. microbe bodies	17	3	-	2	12	2	4	3	2
15th day	440-660 min. microbe bodies	8	1	-	1	7	1	3	3	-
30th day	43-63 min. microbe bodies	20	9	1	3	7	2	3	2	-
30th day	440-660 min. microbe bodies	44	12	-	8	24	12	6	6	-
90th day	43-63 min.	26	4	-	5	17	7	4	6	-
	440-660 min.	26	3	1	4	18	10	5	2	1

It is seen from the table that persons, immunized with a dose comprising hundreds of millions of microbe bodies, had negative allergic reactions somewhat rarer in comparison with those who received tens of millions of microbe bodies. These data are entirely in keeping with the results of tests of effectiveness of the aerogenic anthrax vaccine in crucial experiments with sheep. Animals, vaccinated with a dose of 500-700 min. microbe bodies, as a rule, acquired an immunity of high intensity to the infection of the virulent culture B. anthracis in doses 10-10,000 MLD.

Thus, the dry aerogenic vaccine against anthrax from

strains STI-1 and No 3, while being "areaktogennoi" [producing no reaction], provided an immunological reorganization in the organism of vaccinated persons; these displacements were registered in the dynamics by means of an intracutaneous test with the anthrax allergen.

It was again convincingly shown, on the basis of the conducted work, that it is possible, if necessity arises, to immunize quickly a large number of people with the aid of the aerogenic method of vaccination. Thus, in a 40 m^3 size room, during the course of one hour, we succeeded in vaccinating up to 300 persons with an exposure of five minutes. Whereupon, besides the physician only two workers from among the secondary medical personnel took part in this work. Consequently, having three small rooms, size of 40-50 m^3, or tents of a corresponding size, a brigade of five-six men can vaccinate up to 1,000 persons and more during the course of one hour.

The cited data show that the aerogenic vaccination permitted to vaccinate a comparatively large number of people in a short time; this is especially important in urgent anti-epidemic measures. All this permits to consider the aerogenic vaccination as a rapid method of immunization of people and of agricultural animals, according to epidemiological and epizootiological indications.

Conclusions

1. Dry aerogenic vaccine against anthrax, produced from strains STI-1 and No 3, is virtually areactogenic [producing no reaction] in reasonable doses.
2. Aerogenic immunization with antianthrax vaccine provides an expressed immunological reorganization in the organism of vaccinated persons, which is registered in the dynamics with the aid of the anthrax allergen MIEMG.
3. Aerogenic vaccination can be utilized as a rapid method of immunization according to epidemiological indications.