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**STUDY OF THE REACTOGENICITY, SAFETY,
AND IMMUNOLOGICAL EFFECTIVENESS OF THE
AEROSOL METHOD OF IMMUNIZATION AGAINST
ANTHRAX IN TEXT WITH HUMAN SUBJECTS**

N. E. Gefen, et al

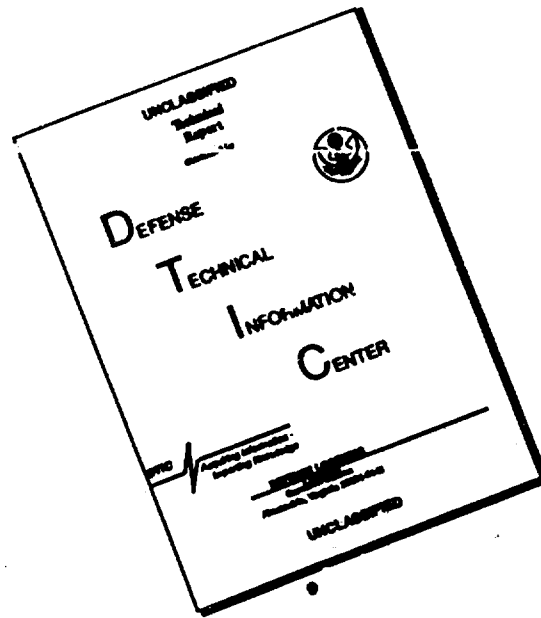
**Foreign Technology Division
Wright-Patterson Air Force Base, Ohio
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Block	Italic	Transliteration	Block	Italic	Transliteration
А	<i>а</i>	A, a	Р	<i>р</i>	R, r
Б	<i>б</i>	B, b	С	<i>с</i>	S, s
В	<i>в</i>	V, v	Т	<i>т</i>	T, t
Г	<i>г</i>	G, g	У	<i>у</i>	U, u
Д	<i>д</i>	D, d	Ф	<i>ф</i>	F, f
Е	<i>е</i>	Ye, ye; E, e*	Х	<i>х</i>	Kh, kh
Ж	<i>ж</i>	Zh, zh	Ц	<i>ц</i>	Ts, ts
З	<i>з</i>	Z, z	Ч	<i>ч</i>	Ch, ch
И	<i>и</i>	I, i	Ш	<i>ш</i>	Sh, sh
Я	<i>я</i>	Y, y	Щ	<i>щ</i>	Shch, shch
К	<i>к</i>	K, k	Ъ	<i>ъ</i>	"
Л	<i>л</i>	L, l	Ы	<i>ы</i>	Y, y
М	<i>м</i>	M, m	Ь	<i>ь</i>	'
Н	<i>н</i>	N, n	Э	<i>э</i>	E, e
О	<i>о</i>	O, o	Ю	<i>ю</i>	Yu, yu
П	<i>п</i>	P, p	Я	<i>я</i>	Ya, ya

* ye initially, after vowels, and after ъ, ь; e elsewhere.
 When written as ѣ in Russian, transliterate as yě or ѣ.
 The use of diacritical marks is preferred, but such marks
 may be omitted when expediency dictates.

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AEROSOL METHOD OF IMMUNIZATION AGAINST
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(Received 7 April 1969)

Over a period of years a high-speed method has been developed for aerosol immunization against anthrax, ensuring rapid creation of a maximally broad immune layer. The research resulted in preparation of a dry aerosol anthrax vaccine whose operational base is live spores of *Bac. anthracis* (STI-1). The vaccine was studied exhaustively in animal experiments and with human subjects. During aerosol immunization against anthrax the same laws governing immunity formation were determined as with subcutaneous and cutaneous [skin] introduction of this vaccine. It was established in experiments on guinea pigs, rabbits, ewes, and monkeys that aerosol immunization imparts to an animal a high degree of specific resistance the virulent culture of anthrax.

As the result of numerous studies, a dry live anthrax vaccine intended for aerosol applications was obtained under conditions of experimental production. At present the optimum recipe

form of the preparation and conditions for its effective application have been determined. The vaccine applied for immunization contained 50-90 billion spores per gram. The positive results obtained during study of aerosol immunization with the vaccine in animal experiments made it possible to move to testing this method on limited groups of people (64 volunteers) in 1957. Preliminary data on the effectiveness of aerosol immunization against anthrax obtained in 1958-1959 were submitted to the Committee on Vaccines and Serums, which announced that the materials presented indicated that the aerosol anthrax vaccine was safe, weakly reactogenic, and effective; the committee authorized its testing with first 500, and then 5000 human subjects.

Testing of the preparation on large groups of people was carried out in two stages: in 1959-1960 (1718 persons) and in 1965 (3517 persons).

In order to obtain comparative data the groups of people were inoculated subcutaneously and cutaneously and also by inhaling neutral dusty material (carrier). The dry live aerosol anthrax vaccine was used for human immunization in doses of 12 to 600 million spores.

The preparation was dispersed by means of atomizer instruments of types UAV and PAV-65; they created aerosol concentrations required to ensure immunizing doses within close quarters.

The reactogenicity was determined according to a widely accepted procedure consisting of medical examination of the inoculated groups, clinical investigation of blood and urine, X-ray study of the organs of the chest cavity, and electrocardiography. Medical observation lasting two weeks was established for the inoculated individuals. As the observations showed, not one of the individuals inoculated by the aerosol method manifested any clinically expressed reaction. The subjective

state of health of the inoculees remained fully satisfactory. Pulse rate and respiration after a 15-minute session of aerosol immunization remained unchanged.

Subclinical reactions manifested as short-term (within 7 days) moderate neutrophilic leukocytosis (to 8500-11,000 per 1 mm³) and in elevation in erythrocyte sedimentation reaction (ESR) were noted in inoculated individuals treated both with aerosol and subcutaneous or cutaneous vaccination. Investigation of hematological changes in inoculated individuals with the magnitude of the inhaled dose revealed a direct dependence of subclinical reactions on the magnitude of aspirated dose of aerosol anthrax vaccine. Thus, in persons inoculated with 50 million spores leukocytosis was established in 33% of the cases; in individuals receiving 300 million spores leukocytosis was noted in 95.8% of the cases, while in subcutaneously inoculated subjects the rate was 20%. Acceleration of the ESR was noted only in individuals aerosol-inoculated with 300 million spores (20% of the subjects). As a rule, acceleration of ESR was recorded on the 3rd day after aerosol vaccination. It was not possible to establish any essential differences in hematological shifts in individuals inoculated once and those inoculated twice.

During X-ray observation of aerosol-inoculated individuals it was not possible to detect any pathological changes in the lungs and regional lymphatic nodes in any case. Analysis of electrocardiograph data of inoculated individuals also revealed no essential deviations in cardiac activity.

In the control groups people who inhaled the neutral carrier dust included in the composition of the vaccine also showed no deviations from normal.

In evaluation of the safety of the aerosol immunization method there is considerable interest in the results of a 5-year

committee observation over a professional group of people inoculated by aerosol methods against anthrax. A total of 1186 workers and employees of the Kishinev Meat Combine and Leather Plant were placed under medical observation. Medical specialists carried out daily dispensary examinations of inoculated and control groups. Exposure of current morbidity was ensured by physicians at health stations. The investigated materials attested to the fact that aerosol immunization did not have any negative influence on the state of health of the inoculated individuals. Over the course of 5 years the frequency of primary treatments was identical for the inoculated group and the control group. Analysis of the structure of primary treatment requirements in terms of individual nosological forms showed a basic similarity in the nature of morbidity in the two groups. In particular, aerosol immunization did not cause any increase in the number of pulmonary and allergic illnesses in the inoculated group.

The immunological effectiveness of the aerosol immunization method against anthrax was studied in comparison with subcutaneous and skin inoculations with STI vaccine by means of skin-allergic tests with anthraxin prepared at the Moldavian Institute of Epidemiology, Microbiology, and Hygiene. We undertook repeated efforts to use certain serological laboratory methods to clarify the immunological reaction of the organism to a specific vaccine; however, as yet none of them have given positive results. Titration of antibodies by means of diffusion precipitation with an antigen is not an adequately reliable and sensitive method. The fact that no antibodies have been found in the blood serum of a significant portion of the inoculated individuals by means of diffusion precipitation in gel remains unexplained. A vast amount of research on the preventive properties of serum from inoculated individuals and also on the reaction of passive hemagglutination, have not always reflected the state of immunity of the inoculated individuals.

The simplest and most reliable method, which has found favor with many immunologists, turned out to be intradermal allergic tests with anthraxin.

Data obtained in 1959-1960¹ showed that in subjects (1718 persons) inoculated against anthrax (doses of 12-15, 40-60, 400-600 million spores) subcutaneously and cutaneously showed a rapid growth in the number of positive allergic reactions over the first two weeks of observation (see table). This attested to the occurrence of immunological shifts in the organism of the inoculated individuals. After a month the number of positive responses in all groups was somewhat reduced, a fact which has not as yet found satisfactory explanation. During subsequent observations over the course of 3 months a certain increase in the number of positive responses was noted, with gradual damping of skin-allergic reactions over the course of a year. It should be noted that in this experiment it was found that the number of positive reactions depended on the dose of vaccine. Thus, with aerosol introduction of 15, 60 and 600 million spores after a month the number of reactions was expressed, respectively, as 23, 56, and 55%; with subcutaneous vaccination it equaled 39% and with cutaneous application, 27%. The dependence did not have a linear nature and was smoothed with approach to the physiological limit of reactivity of the vaccinated individuals. Thus, 3 months after vaccination with a dose of 60 million spores the number of positive reactions equaled 56%, while with a dose of 600 million spores it equaled 55%. The obtained data clearly indicated that a dose of 40-60 million spores is optimum for aerosol immunization against anthrax. Increasing the dose by as much as ten times did not lead to any noticeable change in the intensity of the allergic reaction. With subcutaneous and,

¹Together with Shlyakhov and Tamarin (Shlyakhov, 1968).

especially, cutaneous vaccination a smaller number of positive allergic reactions were noted.

Results of skin-allergy tests with anthraxin in individuals immunized with dry live anthrax vaccine in the form of an aerosol and with subcutaneous and cutaneous inoculation with STI vaccine.

Day of obser- vation	Immunization method														
	Application as aerosol									Subcuta- neous		Cutaneous			
	2-15 mln. spores			40-60 mln. spores			440-660 mln. spores			50 mln. spores		2 drops of spore sus- pension			
	Number of subjects	Number of positive reactions (as %)	Index of reaction intensity (as %)	Number of subjects	Number of positive reactions (as %)	Index of reaction intensity (as %)	Number of subjects	Number of positive reactions (as %)	Index of reaction intensity (as %)	Number of subjects	Number of positive reactions (as %)	Index of reaction intensity (as %)	Number of subjects	Number of positive reactions (as %)	Index of reaction intensity (as %)
15-th	111	57.7	18.0	17	70.6	11.1	9	78.0	11.4	49	47.0	22.2	48	46	24.7
30-th	119	33.6	11.8	34	50.0	27.6	45	55.5	21.7	46	39.2	22.0	66	27.3	13.3
90-th	221	13.6	20.3	56	35.0	33.6	26	69.1	23.3	50	34.0	27.5	37	35.1	12.5
180-th	179	11.4	13.2	35	32.5	12.1	10	10.0	17.5	102	37.3	15.8	98	27.6	12.7
365-th	150	25.3	9.4	24	37.4	14.1	38	34.2	17.1	90	24.5	11.4	91	25.1	11.9
Not inocu- lated	755	4.3	1.76												

Study of the immunological effectiveness was continued to 1965. In this experiment an improved instrument for aerosol vaccination was first used to immunize humans - the PAV-65, permitting more economic use of vaccine. Immunization was carried out in single and double doses of 50 and 300 million spores. A total of 3517 persons were aerosol-vaccinated and 300 subjects were vaccinated cutaneously with SPI vaccine. The effectiveness of immunization, as in the first experiment, was monitored by study of skin-allergic reactions to the introduction of anthraxin into the inoculated individuals (anthraxin series No. 21 and 23). Comparative data on the immunological effectiveness of aerosol and cutaneous vaccination were obtained from counting 671 reactions in individuals aerosol-vaccinated, 172 reactions in cutaneously vaccinated subjects, 328 reactions in subjects which inhaled a neutral substance - imitation - and 28 non-

inoculated subjects. We were concerned with the question of the degree to which the number of positive reactions with anthraxin depends upon the magnitude of the inhaled dose, the method of immunization, and the number of vaccinations. Inoculated subjects were observed for a period ranging from 3 days up to 3 months after vaccination.

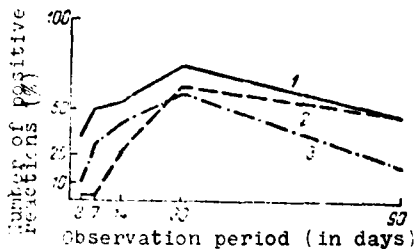


Fig. 1.

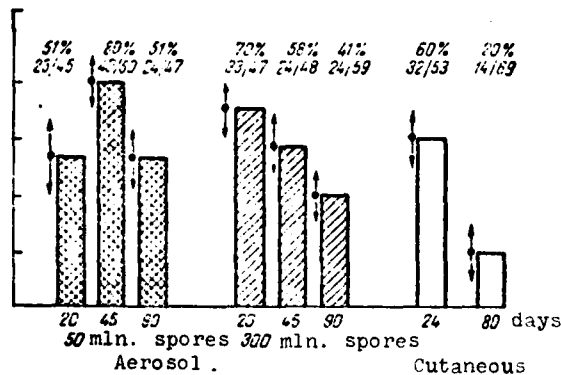


Fig. 2.

Fig. 1. Dynamics of skin allergic reactions with anthraxin in individuals immunized by the aerosol method with dry live anthrax vaccine and individuals vaccinated cutaneously with STI vaccine.

1 - aerosol, dose 300 million microbe cells; 2 - aerosol, dose 50 million cells; 3 - cutaneous (~200 million microbe cells).

Fig. 2. Immune-allergic indices in persons vaccinated with dry live aerosol anthrax vaccine under conditions of mass vaccination.

As far as can be judged from the obtained data (Fig. 1), the vaccine doses utilized caused immune reconstruction in the organism of inoculated individuals as early as the 3rd day after vaccination. The greatest number of positive reactions was noted in the period from the 15th to the 45th days of observation. In this span reactions reached maximum intensity and comprised 28-63 and 58-70% in groups aerosol inoculated with 50 and 300 million spores, respectively, and 44-61% in the skin-inoculated individuals (Fig. 2). After 3 months the number of positive reactions in subjects inoculated by the aerosol method was reduced to 40-50%, while in the skin inoculated group it

fell to 20%. In this experiment, as in the first, we were unable to note any damping of the reaction within a month after vaccination. The dependence of the immune allergic reaction on aerosol vaccine dose was expressed as a somewhat slower rise in the reaction within the first two weeks from a dose of 50 million spores as compared with a dose of 300 million spores (see Table [sic] 2). In this case the number of positive reactions for the 50-million spore dose comprised 28% and that for the 300-million dose was 35%. However, with subsequent observation between the first and third months after vaccination the number of positive reactions in both groups leveled out to identical values - 47-52%. In this experiment we were able to confirm earlier data indicating that 50 million spores make up the optimum dose.

The index of reaction intensity was found to be dependent on the period of observation, reaching maximum indices 14-30 days after vaccination; for the 50-million dose it comprised 37.5% and for the 300 million dose, 32.3%; with cutaneous vaccination it was lower, equaling 24.5%. The number of vaccinations had essentially no influence on either the number of positive reactions or on reaction intensity. It is important to note that the number of nonspecific reactions to intradermal introduction of chemical anthrax in noninoculated individuals and individuals inoculated with the imitation averaged 5%. The number of nonspecific reactions showed no tendency toward growth and essentially was not reflected in the evaluation of immune-allergic readjustment of individuals vaccinated against anthrax by the aerosol or cutaneous method.

Conclusions

1. By means of skin-allergic tests with anthrax it is possible to provide reliable documentation of the immune-allergic readjustment of the human organism to the introduction of anthrax both by the aerosol and the skin method.

2. Immunization against anthrax with dry live aerosol anthrax vaccine is safe, nonreactogenic, and not inferior in effectiveness to the subcutaneous vaccination method: it is somewhat superior to the cutaneous method, as indicated by experiments on animals and observations of human subjects (5232 individuals).

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