DRY, LIVE, AEROSOL ANTHRACIC VACCINE

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ABSTRACT:

Production of a live, dry, aerosol anthrax vaccine, from anthrax vaccine strain STI-1 spores, as well as differences from previous methods of production, are described. A 15 minute aerosol immunization session, in the presence of 0.05 g per m^3 concentration of vaccine is said to insure inhalation of 1 immunizing dose of anthrax spores. Determination of storage requirements for preservation of spore viability is discussed.

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As a result of research over many years on development of the aerosol method of immunization against anthrax, a dry, live, aerosol vaccine has been produced, which has been subjected to comprehensive study in extensive experiments on animals and limited groups of people.

The results of development of the aerosol method of immunization with anthrax vaccine confirm the necessity for further study of the harmlessness, reactogenicity and effectiveness of this method of immunization, as well as the necessity for improvement in the methods of producing aerosol vaccines with high biological acitivity and good aerosol dispersion properties, for immunization of large groups.

The results of research on improvement of dry, live, aerosol anthrax vaccine carried out in 1965 are presented in this report.

A spore culture of one anthrax vaccine strain (STI-1) in place of a mixed culture of two strains (STI-1 and No. 3), was used for manufacture of the preparation, which significantly simplified the technological process.

Culture of anthrax vaccine strain STI-1 was carried out in the Shesterenko microbe culture apparatus (SMCA), which insured the production of large quantities of spore culture.

A 1-day culture of anthrax vaccine strain STI-1 grown in mats at 34°, was used as the inoculum. 1-2 mats of 1-day culture of strain STI-1, washed with physiological solution, was diluted to 5 l with physiological solution and, after testing for purity, nature of growth, pH and microbe cell concentration (by an optical standard), it was inoculated onto an agar medium in SMCA.

Immediately after inoculation with the mother culture, the automatic aeration system was switched on and the apparatus was set at a specific inclination to the vertical axis, 7°, for the first 2 days, and at 60° during the remaining culture time.

Air was supplied to the apparatus during the entire culture period (5 days), at a rate of 25 l per min, for 20 min every 2 hours. In this aeration mode, the spore culture harvest, both by testing according to the optical standard and by number of spores per ml of wash, was higher than aeration only for a period of 2 days. The culture temperature was maintained at a level of 34-35°.

The culture conditions described insured the production of large quantities of spore culture (6-6.5 l from one charge of the apparatus), with a concentration of 3-10 X 10^9 spores per ml, of which 70-80% were viable.

Anthrax vaccine strain STI-1, both on mats and in the SMCA, were cultured on agar media, the base of which was a tryptic hydrolyzate of meat or fresh fish.

For the purpose of increasing the concentration of spores per g of dry vaccine, distilled water was used for washing the production spore cultures from the SMCA and as the drying media, in place of the previously used stabilizer (a mixture of sugars with peptone). In this case, the spore content per g of dry vaccine was increased 2-3 times (50-87 X 10^9/g) over the vaccine prepared from stabilizer (20-30 X 10^9/g), and the quantity of viable spores was not reduced in the prepared vaccine.

An inert polydispersion substance (filler) was included in the composition of the aerosol vaccine, to provide the necessary aerial dispersion properties to the latter. The spore culture, washed from the agar medium in the SMCA with distilled water, was impregnated with filler, in the amount of 100-150 X 10^9 spores per g of filler, after which the spore culture with filler was frozen at a temperature of 45° below zero, and then dried in flasks, in a KS-6 drying unit, for a period of 24-26 hours. Drying was carried out under vaccuum (at the beginning of drying, at a level of 250-400 μ, and 100-180 μ of mercury at the end), the cartridges were heated to 40° above zero for the first 8-10 hours, and to 30° above zero, until the end of drying.

The entire dry, live, aerosol anthrax vaccine production cycle lasted 7 days (1 day, preparation of inoculum; 5 days, culturing in SMCA; 1 day, freezing and drying of the vaccine material).

The use of the model PAV-65 with the aerosol vaccine insured dispersion of the dry, live, aerosol anthrax vaccine at the moment of atomization, and it permitted preliminary grinding of the preparation in a grinding mill to be eliminated, which facilitated production of a vaccine free of foreign microflora.

Dispersion of the vaccine at the moment of atomization permitted a highly dispersed vaccine aerosol to be created, owing to which the vaccine use factor increased and the batch size dispersed per m^3 of space decreased.

For a comparative evaluation of the suitability of different series of the aerosol immunization preparation, a "dispersibility criterion" was established experimentally: the size of the calculated aspiration dose of the preparation (aerosol concentration in 150 l of air by dispersion of a 0.05 g per m^3 batch of vaccine with the PAV-65 instrument) should amount to at least 1 mg. Under these conditions, a 15 minute aerosol immunization insures inhalation by a person (the pulmonary ventilation volume equals 10 l on the average) of not less than 1 immunizing dose (50×10^6) of anthrax spores.

A study of all series of dry, live, aerosol anthrax vaccine, produced by the process described above, showed that the calculated aspiration dose of the preparations, in which distilled water was used as the drying medium, amounted to more than 1 mg, i.e., by the "dispersibility criterion", these preparations were suitable for aerosol use.

The storage conditions of the preparation (degree of vaccuum and temperature) are of great importance for the duration of spore viability preservation in the dry, live, aerosol anthrax vaccine.

In the absence of vacuum, the content of live spores in the preparation, in ampules or flasks of the vaccine, decreased after 6 months of storage in an indoor refrigerator. An appreciable decrease was observed in the live anthrax spore content in a vacuum, in flasks and ampules of the vaccine, in the event the preparation was stored at 20° above zero.

Conclusions

1. In production of dry, live, aerosol anthrax vaccine, the use of a spore culture of 1 vaccine strain STI-1 and culture of latter in a solid media in a SMCA significantly simplified the culture process and insured production of large amounts of spore culture, with concentrations of up to 10×10^9 spores per ml of wash.

2. Dry, live, aerosol anthrax vaccine is suitable for aerosol immunization, if the calculated aspiration dose, by dispersion of a 0.05 g per m^3 vaccine batch with the PAV-65 apparatus amounts to at least 1 mg. Under such conditions, inhalation by a person of at least 1 immunizing dose of anthrax spores is insured in a 15-minute aerosol immunization session.

3. For prolonged preservation of viable spores in the dry, live, aerosol anthrax vaccine, it must be stored under conditions of high vaccuum (100-150 μ) and at a temperature of no more than +10°.