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SEARCH FOR EFFECTIVE VACCINES AGAINST CERTAIN ZOONOZES.

COMMUNICATION I.

PREPARATIONS OF ANTHRAX CHEMICAL VACCINE AND THE STUDY OF ITS EFFICACY IN EXPERIMENTAL ANIMALS.

N.I. Alexandrov, N.E. Gefen, A.P. Budak, Yu. V. Ezepchuk, A.I. Filippenko and V.F. Runova.

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In our preceding studies (Alexandrov and Gefen 1956, 1957, etc.) we defended and developed the feasibility and necessity of preparing effective chemical vaccines against a number of infections, i.e., plague, tularemia, brucellosis and anthrax, although this type of vaccination against the above infections had been considered unrealistic in the recent past. The above-mentioned studies depicted certain methods for the research of these vaccines. Gladstone (1946) was the first scientist who developed the protective anthrax antigen; he cultivated the B. anthracis on sheep blood plasma nutrient medium. Somewhat later Wright et al., proposed a synthetic medium for the cultivation of the antigen; the medium consisted of 16 amino acids; purinic and pyrimidinic bases, vitamin B, glucose and mineral salts. Subsequently, this medium was somewhat altered (Puziss and Wright). The production of antigen on more complicated media, consisting of hydrolised caseinic acid, was described by Belton and Strange, Thorn and Belton, Strange and Belton.

Different methods were applied for the purification and concentration of the anthrax antigen: Precipitation with alumino-potassium alums (Wright et al., Jackson, Wright and others); sedimentation with acids and alcohol (Boor and Tresselt); fractionation with ammonium sulfate (Thorn and Belton).

Recently in the U.S.A., a number of articles was published - depicting the high immunizing effectiveness of a chemical anthrax vaccine, well-tested on monkeys, sheep, and large horned cattle. In addition, a substantial number of studies has been published; these studies confirmed the absence of severe reactions and quoted the high immunologic effectiveness of the chemical anthrax vaccine, when tested on persons. A massive production of the chemical anthrax vaccine intended for the use on people - exists in the U.S.A.

In our first experiments, in order to develop an extracellular preventive antigen, the cultivation of the anthrax bacterium was effected on the synthetic medium, as proposed by Wright (1954). We also succeeded in developing an effective anthrax antigen, by employing this medium; this confirmed the American data. Nevertheless, the excessive costs of the components of this nutrient medium, i.e., amino acids and purinic bases, - forced us to

search for a less expensive and practicable nutrient medium. Our investigations discovered a medium which consisted of skim-milk, glucose and a number of nonorganic salts, aiding the preparation of an anthrax antigen.

In addition to the development of an inexpensive and accessible nutrient medium, we were faced with the necessity of selecting the most effective prophylactic antigen from a number of vaccinal anthrax strains. For this purpose we tested the following strains: i.e., B. anthracis,- standard vaccinal strain STI - I (without capsule, non-proteolytic); standard vaccinal strain STI - 374 (without capsule, non-proteolytic); standard vaccinal strain STI - 353 (without capsule, non-proteolytic; vaccine STI of the Vaccinal strain STI-1 (Kaluzh Bio-Plant) and vaccine STI of the Tiflis Institute for vaccines and sera prepared from a suspension of two vaccinal strains - STI-1 (without capsule, non-proteolytic) and # 3 (without capsule, proteolytic).

The capabilities of the enumerated strains to manifest a specific protective antigen, when grown on our developed nutrient medium, was studied by means of the quantitative determination in the cultural liquid, by employing a diffused precipitation test on agar (Ouchterlony). In staging this test, for anti-serum qualitative purposes, we always employed the same therapeutic anti-anthrax serum. The obtained data showed that out of all the tested strains, the most effective antigen was developed from the vaccinal strain STI-1 (Kaluzh Bio-Plant vaccine): the titer of the antigen in the cultural liquid of this strain = 25 units whereas the standard strain STI-353 and Vaccine STI = 20 units; and standard strains STI-1 and STI-374 only 15 units. Hence we used this strain (STI-1) in all our subsequent experimentation. For cultivation - we used a 24-hour culture of the strain and planted - 5000 spores per 1 ml of nutrient medium. The inoculated medium (in test tubes) was kept at 37° for 20 to 24 hours; subsequently, the remaining casein - unused by the anthrax bacteria - was precipitated in an isoelectric device with the pH = 4.5 to 4.7. The sediment consisted of non-specific albuminous elements and microbic cells; the specific protective antigen was left in the unsedimented liquid; the quantity was determined by means of the diffused precipitation test in agar. We employed the precipitation test with alumino-potassium alums in order to isolate the specific protective antigen from the unsedimented liquid. The protective antigen, precipitated by alums was effected by an addition of 0.1% alum to the unsedimented liquid with a pH = 5.9. The sediment of the precipitated antigen was separated by centrifuging and subsequent desiccation.

The alumino-potassium alums - in this instance - were not only the adsorbent of the protective antigen, but also the stabilizing factor of the labile protective antigen; in addition, the function of the sedimentative element was performed during vaccination by its adsorbing antigen.

At the beginning, the effectiveness of vaccination was studied on white mice. Various doses of the antigen - adsorbed by alum - were injected into the animals, subcutaneously - once, twice and three times - at various

intervals. After seven days, following the last vaccination, we determined the immunity, which had developed in the animals, by means of establishing their specific resistance to an infection with lethal doses of anthrax spores of vaccinal strains (it was known that these had retained a sufficiently high virulence for small laboratory animals).

The data expressed in Table 1 and 2 indicate the effectiveness of the chemical anthrax vaccine in relation to the vaccinal dose used for immunization. The employed optimum doses of the chemical anthrax vaccine created a clearly pronounced specific resistance in laboratory animals. Here the most effective appeared to be - two injections at seven day intervals. The harmlessness of the chemical anthrax vaccine was studied on white mice and rabbits, and the manifestation of reactions - on monkeys. For this purpose varied doses of vaccine were injected (into white mice and rabbits), intraintestinally; and into monkeys - subcutaneously. The experimental animals were observed carefully for seven days. Table 3 indicates that the vaccine was safe for mice: with injections = 25 mg., all mice survived. Rabbits were injected with doses = 100 to 250 mg; and all survived. Two monkeys injected with 100 mg. manifested no local, nor general reactions; three monkeys injected with 400 mg. manifested a weak reaction - in one monkey evidenced on the 5th to 7th day; in another on the 7th day.

In addition to the tests staged on small laboratory animals, we studied the immunizing effectiveness of the anthrax vaccine in specific tests on large animals - sheep. A total of 45 sheep was used for this experimentation. Out of these, 15 were vaccinated subcutaneously with the adsorbing chemical anthrax vaccine; 18 were vaccinated with live anthrax vaccine STI and 12 were used as control animals. Twenty days after vaccination, the immunized, as well as the control sheep, were infected intra-cutaneously with massive doses of spores of the highly virulent strain B. anthracis #836.

The data shown in table 4 evidence that a repeated immunization of sheep with chemical anthrax vaccine produced a rather strong immunity; the immunity protected them from an infection caused by a massive dose of the highly virulent anthrax strain. The immunity developed after the two injections with the chemical anthrax vaccine did not seem to be inferior to that induced when the animals were injected with live anthrax vaccine STI.

CONCLUSIONS.

1. A comparatively simple and inexpensive method was developed for the preparation of a chemical anthrax vaccine adsorbed by alumino-potassium alums.

2. The data produced on experimental animals evidenced that the vaccine was safe, caused no reactions and appeared to be highly effective.

3. It would be expedient to conduct more extensive and intense studies of the chemical anthrax vaccine, by immunizing large farm animals, and humans.

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Table 1.

DETERMINATION OF OPTIMUM IMMUNIZING DOSE OF CHEMICAL ANTHRAX VACCINE ON WHITE MICE WITH SUBSEQUENT INFECTION OF SEVEN DAYS - 2.5 bln. SPORES OF VACCINAL STRAIN STI-1.

Nomenclature of immun. prep.	Vacc. dose (mg)	# of imm.	Interval (days) Between 1st and 2nd vac.	Betw. 2nd and 3rd	# of Mice Exp.	Survived
Chemical anthrax vaccine, precip. with alums	25	3	1	7	30	12
	50	3	1	7	130	130
	80	3	1	7	21	21
Control (non-immunized)	-	-	-	-	40	0

Table 2.

DETERMINATION OF IMMUNIZING POWER OF CHEMICAL VACCINE ON WHITE MICE WITH VARIOUS METHODS USED AND INFECTION SEVEN DAYS LATER WITH 2.5 bln SPORES OF VACCINAL STRAIN STI-1.

Nomenclature of immuniz. preparation	Vacc. dose (mg)	# of imm.	Intervals (days) Betw. 1st and 2nd	Bet. 2nd and 3rd	# of Mice Exp.	Survived
Chemical anthrax vaccine, precip. with alums	50	3	1	7	75	75
	50	3	1	3	55	53
	50	2	7	-	670	670
	50	2	3	-	35	31
	50	2	1	-	25	10
	50	1	-	-	25	2
Control (non-immunized	-	-	-	-	50	0

Table 3.

DETERMINATION OF TOXICITY OF THE CHEMICAL ANTHRAX VACCINE ON WHITE MICE

Nomencl. of tested preparation.	Quant. Injec. (in mg)	# of exp. mice	Results of observation (in days) 1st	2nd	3rd	4th	5th	6th	7th	# of survived mice
Chemical anthrax vaccine	80	10	0/10	0/10	2/8	2/6	0/6	0/6	0/6	6
	50	10	0/10	0/10	0/10	1/9	0/9	0/9	0/9	9
	25	10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10
	10	10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10
physiological solution	0.5 ml.	10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10

Specification: numerator - number of lost mice; denominator - number of surv. mice.

Table 4.

DETERMINATION OF IMMUNIZING POWER OF THE CHEMICAL ANTHRAX VACCINE IN SHEEP WITH AN INFECTION ON THE 30TH DAY FOLLOWING AN IMMUNIZATION WITH 100.000 SPORES (100 Dcl) OF THE HIGHLY-VIRULENT STRAIN #836.

Nomenclature of immuniz. vacc.	Dose	# of vacc.	Interval betwen inject. (in days)	Number of sheep tested	Number of sheep Survived	Day of Death
Chemical anthrax vaccine precipitated by alums	600 mg.	2	30	15	14	2nd
Live anthrax vaccine STI-1	1 hum. dose	1	-	18	15	5th, 6th, 14th
Control (non-imm.)	-			12	0	2-3rd