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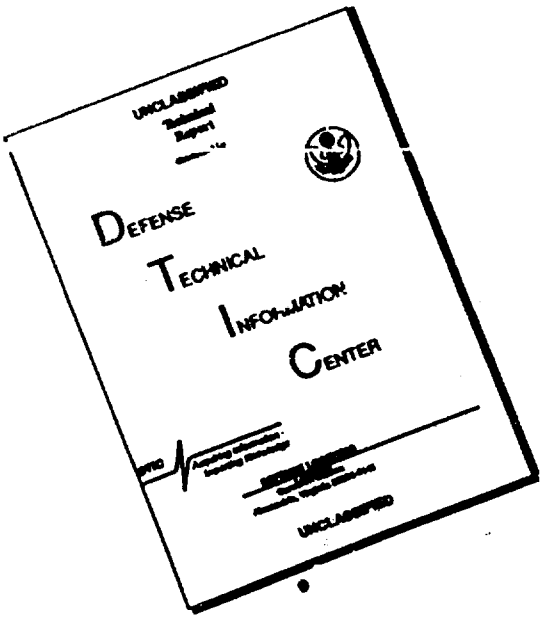

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MATERIALS ON ASSESSING THE METHODS OF ANTHRAX LABORATORY DIAGNOSIS AND IDENTIFICATION OF THE CAUSATIVE AGENT

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To corroborate the methods of indication and identification of Bac. anthracis, listed in the Instructions on Examination of Water, Soil and Washings from Objects of the Environment for the Presence of the Anthrax Causative Agent, approved by the Ministry of Health USSR on 12 June 1965, a study was made of 55 anthrax strains of different virulence and of 43 strains of saprophytic, aerobic bacilli (soil bacilli).

As a result of the conducted studies, data have been obtained which characterize all the signal and conclusive methods of indication and identification of anthrax causative agent, within the scope of this investigation.

The signal study methods, which included the rapid biological test for in vivo capsulation (after Shlyakhov and Gruz) and in vitro capsulation on the culture medium of the State Control Institute [1], were shown by our data to be highly sensitive, especially the rapid biological test for capsulation, which was found to be positive for 100% of the virulent strains under study, that is, capsulate bacilli were

[1] We did not test the serological luminescece signal method, since this work was conducted at another laboratory; no determination was also made of the virulence in rabbits.

demonstrated in smears of abdominal cavity contents and in the impression smears of internal organs of the killed mice.

The in vitro test for capsulation is equally reliable for differentiation of virulent, potentially capsulate strains. In this test we utilized, concurrently with the fluid medium of the State Control Institute, the Buza's solid medium (Buza, 1941), consisting of "starved" agar and 15% of fresh sheep blood. Inocula on this medium were incubated for 24 hours in an atmosphere containing 50% CO_2. As a result, round mucoid colonies consisting of chains of capsulate bacilli were obtained with 42 of the 47 tested anthrax strains, i.e. somewhat less consistently than in the fluid medium. The advantage of this medium resides in the fact that its employment demonstrated at the same time the hemolytic and the hemopeptic properties of the bacilli.

While establishing in general a positive assessment of the signal study methods it should be noted that they are not suitable for use in the rare instances when on the environmental objects are present acapsulogenic strains of anthrax causative agent, which has been pointed out by many researchers (Mirotvorskiy, 1940; Abdulin, 1941; Andreyev, 1948; Arkhangel'skiy and Antonov, 1949; Samodelkina, 1950; Kolyakov, 1960; and others). In view of this it is still appropriate to include among the signal methods the test for lysability with a specific bacteriophage.

The conclusive study methods, based on testing of a number of morphological traits and biochemical properties of the microbes, and also on encapsulation in vivo in the form of classical biotest and relationship to a specific bacteriophage, require a pure culture of the isolated microorganism (except for the biotest), and in the test with bacteriophage -- isolated colonies.

The methods appertaining to this study section are characterized by different degrees of sensitivity. Of highest sensitivity, according to our data, are the "pearl necklace" test and the test with a specific phage, since both these tests are applicable also for an indication of acapsulogenic strains, which may not be said relative to tests for capsule formation, including also the classical biotest.

In addition to the procedure recommended in the Instructions for the performance of the "pearl necklace" test (the Instructions refer to it as the test with penicillin) we made use also of a modification of this test, proposed by one of us (Ye. V. Gruz), which is as follows. To beef-peptone bouillon (pH 7.2-7.4) were added under sterile

conditions 30% horse serum intended for preparation of nutrient media, and 0.5-0.05 units of penicillin per 1 ml of medium. The culture medium was distributed, under sterile conditions, in portions of 2-3 ml into the test tubes which were then inoculated, each, with 2 drops of bouillon culture or with one loopful of agar culture of the strain under study. Inoculation into a test tube containing bouillon without penicillin was used as control. The test tubes were kept in a thermostat at 37° for 3 hours, after which smears were made which were fixed with Carnoy's fluid (until the liquid had evaporated), stained with Methylene Blue and microscoped. In the case of a negative result the incubation was continued up to 6 hours.

With all the 49 anthrax strains tested in these experiments a positive result was obtained after 3 hours of incubation, i.e. in the smears were demonstrated spherical forms of anthrax bacilli. In the smears from the control test tubes (containing no penicillin) were observed the usual rod-shaped forms.

It should be noted that following growth in bouillon containing 0.5 penicillin unit per 1 ml of the medium, positive results were obtained in all instances, whereas with a penicillin concentration of 0.05 unit, 8 strains of anthrax grew after 3 hours of incubation as the usual rod-shaped forms. Therefore, in carrying out the "pearl necklace" test we made use of only one concentration of penicillin, namely that of 0.5 unit per 1 ml of medium.

On testing the 42 strains of sporal saprophytic aerobes, in no single instance were any spherical forms obtained, that is the "pearl necklace" test was negative. In the test tubes containing penicillin at low concentration, as well as in the control test tubes, these bacilli grew as the usual rod-shaped forms.

The absolute reliability of the "pearl necklace" test is confirmed in their communications by Jensen and Kleemeyer (1955), Kolyakov and Melikhov (1960) and by Akimovich and Samoylov (1964).

Identification of capsulogenic as well as acapsulogenic strains of Bac. anthracis is possible also with the use of a specific anthrax bacteriophage. The question of isolation of anthrax phages and of phage-identification of anthrax bacillus has been extensively dealt with in the publications of Ivanovics and Lantos (1958), Stamatin (1959, 1963), Stamatin and Anghelesan (1961), Seidel (1962), Zemtsova (1963), and others.

As a anthrax bacteriophage we utilized the original,

specific bacteriophage BA-9, which had been isolated at our laboratory (Ye. V. Gruz).

Specificity of the BA-9 phage was tested on 52 anthrax strains of different virulence, and on 43 strains of sporal saprophytes. Phage-identification was effect by the following procedure. Into Petri dishes with an absolutely level bottom was poured molten 1.5% beef-peptone agar, after the solidification of which the dish-bottom was ruled into squares with sides at least 2 cm long. The dishes were then partially dried in a thermostat, and in each square was written the number of the culture which was deposited at the center of the square by using a large loop (5 mm). To the test were usually subjected 5-6 hour bouillon cultures or any colony, suspended in physiological solution. The dish with a partially opened lid was again allowed to dry in the thermostat for 30 minutes, after which the bacteriophage was applied with a small loop (2 mm) at the center of the dried-out drop. After incubation for 6-8 hours a determination was made of the results (occurrence and extent of lysis of the colonies).

As a result it was ascertained that all 52 strains of anthrax causative agent were phage-positive, that is they were lysed by BA-9 phage, while out of the 43 strains of sporal saprophytes, 42 were phage-negative, and in only one strain of anthracoid was observed some thinning of the growth at the place of deposition of the phage upon the culture.

Morphological tests for mobility and character of the colonies were found to be somewhat less reliable. Thus, while no mobile variant was detected among the tested anthrax strains, out of the 43 saprophytic aerobic bacilli 3 were found to be immobile or of low-mobility (2 strains of Bac. cereus, 1 strain of Bac. pseudoanthracis). Growth of anthrax bacilli on beef-peptone agar was, as a rule, typical (one strain grew as S-form colonies). As concerns the saprophytic aerobic bacilli, 11 strains of Bac. cereus grew as colonies that were almost undistinguishable from those of anthrax. On beef-peptone gelatin, in 4 out of 53 strains of Bac. anthracis was not observed the characteristic growth shaped like a fir tree turned upside down, while 2 out of the 27 strains of sporal saprophytes did not induce gelatin hydrolysis, either on the 3rd or on the 6th day of incubation.

The classical biotest was found to be positive in all cases when it was carried out with virulent anthrax strains (44). Duration of survival of the animals varied from 18 to 48 hours. In tests with vaccine strains and those of a low-virulence, duration of survival of the animals was extended

to 78 hours and longer (in one case the death occurred on the 7th day).

In tests of sporal saprophytic aerobs the classical biotest was found to be negative and only in one case (strain of Bac. cereus) the animals perished within 18 hours following infection, but in the impression-smears of the internal organs no capsulate bacilli were found. A shortcoming of the classical method of effecting the biotest for animal sensitivity to anthrax, in addition to its long duration, is the obtaining of negative results when avirulent, acapsulogenic strains are present.

Conclusions

1. The procedure of laboratory diagnosis of anthrax and identification of Bac. anthracis, proposed in the Instructions on Examination of Water, Soil and Washings from Objects of the Environment for the Presence of Anthrax Causative Agent, was found to be essentially substantiated in practice.

2. Of the signal methods which we have studied the most effective (ready effectuation and reliability in combination with short duration) was found to be the method of in vivo capsulation, and the test for in vitro capsulation on the medium of the State Control Institute, which requires, however, a greater length of time.

3. The most effective conclusive method, on the basis of those which were tested, is the "pearl necklace" test.

4. Determination of specific lysis of bacilli by the bacteriophage, may be regarded as an additional signal method of investigation.

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