

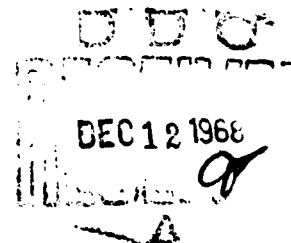
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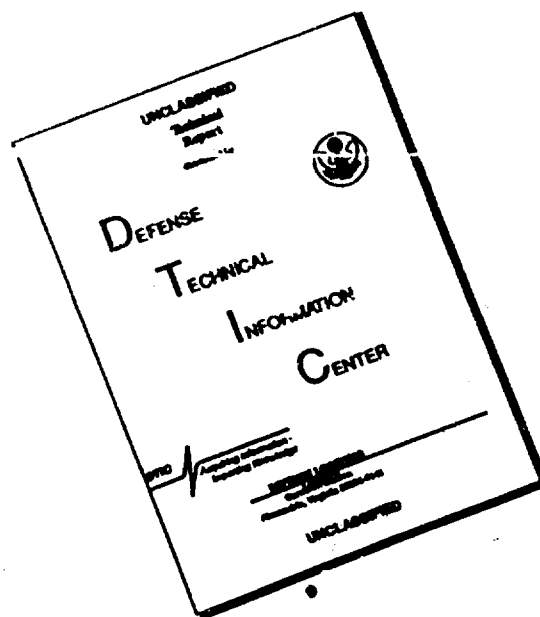


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### OBTAINING OF SPECIFIC ANTHRAX FLUORESCENT SERUM

Following is a translation of an article by V. A. Blagoveshchenskii, A. Ya. Kul'berg, T. I. Bulatova and M. Ya. Korn in the Russian-language journal Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), Moscow, Vol. 33, No. 3, 1962, pages 18-23.

During recent years the luminescent-serological method has been successfully used for the detection of the causative agents of various infectious diseases. The possibility of using this highly sensitive method for a rapid diagnosis depends on how specific the applied serum is, inasmuch as this method is based on the combination reaction of fluorescent antibodies with a corresponding antigen of the microbic cell.

Among a great number of works on the application of fluorescent sera, there are comparatively few studies which treat the obtaining of specific fluorescent sera (Moody, Ellis and Updyke, 1958; Winter and Moody, 1959; and others).

Having started our work on the obtaining and study of diagnostic fluorescent sera, we encountered in many instances sharply expressed cross reactions. Therefore, we were interested, first of all, in clarifying the problem of the possibility of removing these reactions by means of serum adsorption according to Castellani. This work was done with anthrax serum.

The literature which treats the obtaining and the application of anthrax fluorescent serum contains contradictory opinions regarding its specificity. Some authors (Levina, 1958) did not detect cross reactions between anthrax luminescent serum and other microbes, while others (Pritulin and Kuz'min, 1959) have shown the necessity of a preliminary adsorption of the serum according to Castellani by related types of microbes, which sharply increases its specificity.

It is known that anthrax precipitable serum used for diagnosis by means of Ascoli reaction contains antibodies

not only for the homologous microbe but also for the types B. anthracoides and pseudoanthracis which are closely related to it. Therefore, in developing the method of obtaining a fluorescent serum which could be used in practice for diagnosing anthrax, the problem of its strict specificity becomes particularly important.

The labeling of the anthrax precipitable serum (of the Tobol'sk Biological Products Plant) with luminescent a stain was done in three stages: 1) Isolation of the globulin fraction from the whole serum. 2) Combination of the serum globulins with the fluoresceine isocyanate [See Note] 3) Liberation of the labeled globulin from the non-bound fluorochrome.

(NOTE) Fluoresceine isocyanate was synthesized by G. I. Mikhailov (Institute of Chemical Reagents) and was made available to us for this work.)

In performing the first two stages of the work, we followed the methods described previously (Larionov and Kuz'min). The quantity of fluoresceine isocyanate introduced into the reaction with albumin was 5% of the total quantity of the albumin.

In the third stage, the liberation of fluorochrome which did not bind with the albumin was achieved by prolonged (10 -- 12 days) dialysis of the labeled serum against the buffered physiologic solution (pH=8.7). Dialysis was stopped after traces of fluorochrome stopped showing in the dialyzation medium. Our experience of obtaining 18 series of labeled sera for various causative agents has shown that it is possible in this way to get rid of a major part of the non-bound pigment. In this case we did not observe any non-specific or background luminescence in the sera obtained in this way when working with bacterial objects. The above-mentioned method reduces to a minimum the loses of the immune globulin which is observed during the liberation of the serum from the free fluorochrome by means of a repeated reprecipitation of it by ammonium sulfate.

According to the published data (Riggs and co-authors, 1958), the method of liberation from non-bound fluorochrome by prolonged dialysis also assured the obtaining of good results in working with such fluorochrome as fluoresceine isothiocyanate and rhodamin isothiocyanate.

The obtained fluorescent anthrax serum was checked for activity and specificity. We determined the dilution limit of the serum which still produced the luminescence of the vaccine strains STI and the second vaccine of Tsenkovskii.

For the preparation of smears, we used in all cases the washed-off samples of 24-hour old agar cultures. The smears were fixed with alcohol in the course of 15 minutes or over a flame, were processed with various serum dilutions

for 15 minutes at room temperature in a humid chamber and then washed for 15 minutes in the physiologic solution which was changed 2 -- 3 times, after which they were placed in the physiologic solution under a glass cover.

The mounts were examined being magnified 90x x 7x, 90x x 10x under a microscope ML-1 or under an ordinary microscope with a luminescent opaque illuminator OI-17 and condenser OI-18 with a lamp SVDSH-250-3.

The fluorescent anthrax serum (not adsorbed) was found to be active. It caused the luminescence of the STI vaccine strains and the 2nd vaccine of Tsenkovskii when diluted 1:64 (Table 1). However, this serum was not sufficiently specific: it also stained the 4 strains of the anthracoids (1312, 96, 103 and one unnumbered) which we had at our disposal and two strains of pseudo-anthrax microbes. The luminescence of the last two strains was clear (++++) even when serum was diluted 1:64. The luminescence intensity of the anthracoids somewhat lowered as the serum was diluted (strain No. 103 was intensively luminescent only when it was processed with undiluted serum).

The detected cross reactions with the types of microbes which are similar, as far as antigens are concerned, to the anthrax rod compelled us to study the methods of increasing the specificity of the fluorescent anthrax serum. For this purpose we conducted experiments on the adsorption of a fluorescent anthrax serum by living bacilli (anthracoid strain No. 1312 and pseudo-anthrax rod strain No. 16).

For 1 ml of fluorescent serum we took material washed off 10-12 test tubes of a 24-hour-old agar culture. The microbe suspension in the physiologic solution was centrifuged for 10 minutes at 5000 revolutions per minute, the liquid above the sediment was removed, and 1 ml of fluorescent serum was added to the sediment. The latter were mixed and the suspension was kept for 2 hours at 37° and for the night was placed in a refrigerator at 4°. The following day the serum was centrifuged at 5000 revolutions per minute for 25 -- 35 minutes, was separated from the sediment of microbic cells and centrifuged again under the same conditions for 15 minutes. If the serum was not intended for storing over a long period of time, it was possible to use it for work without a sterilizing filtration, taking into account that it had an admixture of merthiolate.

It was established during the experiments that after adsorption the activity of the fluorescent serum in relation to the anthrax vaccine strains diminished noticeably, and its specificity sharply increased. The serum adsorbed by the anthracoid strain No. 1312 caused a luminescence of the anthrax vaccine strains and two pseudo-anthrax vaccine strains in a dilution of 1:8, however even in an undiluted form it did not

Table 1  
Specificity of the Fluorescent Anthrax Serum

| 2) Наименование штамма      | 1) Интенсивность свечения при обработке сывороткой |      |                                             |      |                                             |     |                                                                          |      |
|-----------------------------|----------------------------------------------------|------|---------------------------------------------|------|---------------------------------------------|-----|--------------------------------------------------------------------------|------|
|                             | 3) неадсорбированной                               |      | 5) адсорбированной Bac. anthracoides № 1312 |      | 7) адсорбированной Bac. pseudanthracis № 16 |     | 9) адсорбированной смесью Bac. anthracoides № 1312 и pseudanthracis № 16 |      |
|                             | 4) неразбавленная                                  | 1:84 | 4) неразбавленная                           | 1:8  | 4) неразбавленная                           | 1:4 | 10) неразбавленная                                                       | 1:4  |
| 11) СТИ, серия 9            | ++++                                               | ++++ | ++++                                        | ++++ | ++++                                        | +++ | ++++                                                                     | ++++ |
| 11) СТИ, серия 11           | ++++                                               | ++++ | ++++                                        | ++++ | ++++                                        | +++ | ++++                                                                     | ++++ |
| 13) 2-я вакцина Ценковского | ++++                                               | +++  | ++++                                        | +++  | ++++                                        | ++  | ++++                                                                     | +++  |
| Bac. anthracoides № 1312    | ++++                                               | ++   | 0                                           | 0    | +                                           | 0   | 0                                                                        | 0    |
| Bac. anthracoides 6/№       | +++                                                | ++   | 0                                           | 0    | ++                                          | 0   | 0                                                                        | 0    |
| Bac. anthracoides № 96      | ++++                                               | ++   | 0                                           | 0    | ++                                          | 0   | 0                                                                        | 0    |
| Bac. anthracoides № 103     | ++++                                               | 0    | 0                                           | 0    | 0                                           | 0   | 0                                                                        | 0    |
| Bac. pseudanthracis № 16    | +++                                                | ++++ | ++++                                        | +++  | 0                                           | 0   | 0                                                                        | 0    |
| Bac. pseudanthracis № 104   | +++                                                | ++++ | ++++                                        | +++  | 0                                           | 0   | 0                                                                        | 0    |

14) Обозначения: 0 отсутствие свечения; + - ++++ различная интенсивность свечения.

- Legend:
1. Luminescence intensity during processing with serum.
  2. Name of strain.
  3. Not adsorbed.
  4. Undiluted.
  5. Adsorbed by Bac. anthracoides No. 1312.
  6. Undiluted.
  7. Adsorbed by Bac. pseudanthracis No. 16.
  8. Undiluted.
  9. Adsorbed by a mixture of Bac. anthracoides No. 1312 and pseudanthracis No. 16
  10. Undiluted.
  11. STI, series 9.

- 12) STI, series 11.
- 13) 2nd vaccine of Tsenkovskii.
- 14) Explanation of symbols: 0 absence of luminescence; + - ++++ various intensities of luminescence.

cause of luminescence of all four anthracoid strains.

The serum adsorbed by pseudo-anthrax bacteria caused luminescence of the vaccine strains in a dilution of 1:4, did not stain pseudo-anthrax strains at all, and caused a luminescence of the anthracoids only in an undiluted form (+--+).

These studies have shown that the anthracoid strains and the strains of pseudo-anthrax bacteria which we studied are not identical in regard to the antigens, although, when the serum is adsorbed by pseudo-anthrax bacteria, the luminescence intensity of the anthracoids lowered considerably, while the sera adsorbed by the anthracoids stained the strains of the pseudo-anthrax bacteria in the same dilutions as the anthrax vaccine strains.

The observed regularities have been confirmed by us on three series of serum.

Thus, it has been established that in order to obtain a specific fluorescent anthrax serum it is necessary to carry out its adsorption by the types of microbes which closely resemble the anthrax rod in regard to the antigens. Later we obtained a fluorescent anthrax serum adsorbed by a mixture of an anthracoid (strain No. 1312) and pseudo-anthrax bacteria (strain No. 16). Adsorption was carried out according to the method described above. This serum caused only luminescence of anthrax vaccine strains in a dilution of 1:4, while the strains of the anthracoids and pseudo-anthrax bacteria did not luminesce even when processed by an undiluted serum (see illustration).



**NOT REPRODUCIBLE**

The obtained fluorescent anthrax sera, both the adsorbed ones and the unadsorbed ones, were subjected to lyophilic drying in ampoules of 1 ml each. Their activity did not become lower after drying (Table 2). The specificity of these sera was checked in regard to various heterologous types of microbes: The intestinal rod, *Cl. perfringens* and botulinum (the vegetative and spore form), staphylococci, *Rickettsia prowazeki* and *Rickettsia mooseri*, and the vaccine strains of the *Brucella*. None of the strains exhibited specific luminescence.

Table 2  
Activity of Fluorescent Anthrax Serum Before and After Drying

| 1) Наименование сыворотки                                       | 2) До сушки             |                                      | 3) После сушки          |                                      |
|-----------------------------------------------------------------|-------------------------|--------------------------------------|-------------------------|--------------------------------------|
|                                                                 | 4) Разведение сыворотки | 5) Интенсивность свечения штамма СТИ | 6) Разведение сыворотки | 7) Интенсивность свечения штамма СТИ |
| 8) Неадсорбированная                                            | 1:32                    | ++++                                 | 1:32                    | ++++                                 |
|                                                                 | 1:64                    | ++++++                               | 1:64                    | ++++++                               |
| 9) Адсорбированная антраксом                                    | 1:4                     | ++++                                 | 1:4                     | ++++                                 |
|                                                                 | 1:8                     | ++++++                               | 1:8                     | ++++++                               |
| 10) Адсорбированная антраксом и псевдоадсорбированная палочками | 1:2                     | ++++++                               | 1:2                     | ++++++                               |
|                                                                 | 1:4                     | ++++++                               | 1:4                     | ++++++                               |

- Legend:
1. Name of serum
  2. Before drying
  3. Dilution of serum.
  4. Luminescence intensity of the STI strain.
  5. After drying
  6. Dilution of Serum
  7. Luminescence intensity of the STI strain
  8. Unadsorbed.
  9. Adsorbed by an anthracoid.
  10. Adsorbed by an anthracoid and pseudo-anthrax rods.

By means of the obtained sera we have also studies samples washed from soil cultures where, as it is known, anthracoids occur. The study was done according to the following method: To 5 g of soil taken from vegetable and flower gardens, from the streets, city yards, etc., we added 10 ml of the physiologic solution, shook it, filtered the suspension through a cotton gauze filter, and the filtrate was filtered through a membrane filter No. 3. The latter was placed in petri dishes with meat-infusion agar, the sediment on the filter was rubbed with a glass spatula along the entire sur-



face of the agar and was left to stand 18 to 20 hours at 37°.

We considered it necessary to allow the microbes to grow because sporogenous microbes usually occur in the soils in the form of spores which did not always exhibit clear specific luminescence in our experiments and often did not luminesce at all.

The following day after the inoculation of agar, we usually observe either an abundant growth over the entire surface or a growth of numerous colonies. Two smears were prepared from the material washed off the agar or from individual colonies. One of them was processed with an unadsorbed fluorescent anthrax serum and the other with an adsorbed one. Individual colonies were also isolated from water cultures taken from various sources (Moscow River, Timiryazev Pond and a number of small rivers and ponds in the suburbs of Moscow).

When the smears from the soil cultures were examined under a luminescence microscope, the unadsorbed serum exhibited in all cases a specific luminescence of the rods which were morphologically similar to the anthrax bacteria. No specific luminescence was observed when the mounts from the same washed-off matter were stained by the adsorbed fluorescent serum. In a number of experiments, we added suspensions of the STI vaccine strain to the material washed off the petri dishes. In this case specific luminescence of the bacteria was also observed when the smears were processed by the adsorbed serum.

We succeeded in isolating strains from soil and water samples by cultivating individual colonies which also exhibited specific luminescence when processed with an unadsorbed serum and did not luminesce when processed with an adsorbed serum. Such strains occurred more frequently in the soil. In water they were observed much less frequently (Table 3).

Table 3  
A Study of the Cultures from Soil and Water by Means of a  
Fluorescent Anthrax Serum

| 1) Материал                | 2) Число проб | 3) Результаты обработки флуоресцирующей сывороткой |                           |                                              |                           |
|----------------------------|---------------|----------------------------------------------------|---------------------------|----------------------------------------------|---------------------------|
|                            |               | 4) неадсорбированной                               |                           | 5) адсорбированной                           |                           |
|                            |               | 6) число проб, дающих специфическое свечение       | 7) интенсивность свечения | 8) число проб, дающих специфическое свечение | 9) интенсивность свечения |
| 10) Смывы из посевов почвы | 20            | 20                                                 | ++++                      | 0                                            | 11) Свечения нет          |
| 11) Штаммы из почвы        | 62            | 7                                                  | ++++                      | 0                                            | " "                       |
|                            |               | 12                                                 | ++                        | 0                                            | " "                       |
| 12) Штаммы из воды         | 30            | 1                                                  | ++++                      | 0                                            | " "                       |
|                            |               | 3                                                  | ++                        | 0                                            | " "                       |

[See legend following page]

- Legend:
1. Material
  2. Number of samples.
  3. Results of processing with a fluorescent serum.
  4. Unadsorbed.
  5. Number of samples exhibiting specific luminescence.
  6. Intensity of luminescence.
  7. Adsorbed.
  8. Number of samples exhibiting specific luminescence
  9. Intensity of luminescence.
  10. Washed from soil cultures.
  11. Strains from soil
  12. Strains from water
  13. No luminescence.

#### Conclusions

1. In order to obtain a strictly specific anthrax fluorescent serum, it is necessary to carry out its adsorption by microbes which have common antigens with bacillus anthracis;
2. In order to detect bacillus anthracis in water or in the soil, it is necessary to use a strictly specific adsorbed anthrax serum; and

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## PROBLEMS OF SERUM ADSORPTION FOR LUMINESCENT ANALYSIS

[Following is a translation of an article by N. A. Kuz'min in the Russian-language journal Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), Moscow, Vol. 33, No. 3, 1962, pages 23-27]

Sera used for luminescent analysis are usually those obtained through immunization of animals with microbic vaccines. Many of such sera cause luminescence of microbes of related groups and can be used in diagnostic work only after adsorption.

The problem of serum adsorption for luminescent analysis has not been adequately treated in periodical press, although their preparation has a number of special characteristics as compared to the preparation of adsorbed sera for ordinary agglutination and precipitation reactions. The present paper describes our experiments in the obtaining of the adsorbed precipitating anthrax and paratyphoid sera before and after labeling globulins.

We subjected to adsorption the Gertner horse serum produced in a laboratory, agglutinating Breslau serum of the Moscow Institute of Epidemiology and Microbiology and precipitating anthrax serum of the Tabolsk Plant of Biological Products. At first small quantities of labeled unadsorbed sera were prepared. Common globulins were labeled with fluoresceine isocynate according to Coons. When determining their specificity in an experiment with a great number of homologous and heterologous microbes, it was established that the luminescence of the latter (in the presence of common antigens) differed little in the intensity and type from the luminescence of homologous microbes.

For adsorbing we used strains of those related microbes in which a typical group luminescence was observed. The Gertner serum was adsorbed with *S. typhimurium* Breslau and other representatives of the salmonella of group B, Breslau

serum was adsorbed with microbes of group D, and anthrax serum was adsorbed with Bac. anthracoides and pseudoanthracis.

In order to prepare a microbic mass, 18 -- 24-hour agar cultures were washed off with 0.15 M solution of sodium chloride, thoroughly shaken with beads until a homogeneous suspension was obtained and centrifuged for 5 minutes at 5000 revolutions per minute. Then resuspension and centrifuging were repeated two more times. A thick suspension containing 50 -- 60 milliard microbic cells (according to the turbidity standard) was prepared from the sediment of the washed microbes.

Native sera were diluted before adsorption with a 0.15 M solution of sodium chloride 1:10 and were heated to 37°. Greater dilutions are undesirable because subsequent precipitation of globulins is then hindered.

The adsorption of paratyphoid sera was done in several stages with a mixture of adsorbent microbes. The microbic mass was introduced in a proportion of 1 -- 1.5 milliard microbic cells to 1 ml of the diluted serum. It was thoroughly mixed and allowed to stand at 37°. The exposure of the microbes in the serum lasted until the appearance of agglutinates -- first for about 10-15 minutes, and then gradually getting longer. The last exposure was somewhat longer than the usual period of staining microbes with a labeled serum, but did not exceed 40 minutes. Each time after saturation, the suspension was centrifuged for 5 minutes at 5000 revolutions per minute, and then the serum was decanted and checked in the laminated agglutination reaction. The processing was done 4 to 6 times and was stopped when the control agglutination reaction with adsorbent microbes was negative. After labeling, such serum did not usually cause luminescence of these microbes and it was not necessary to subject it to additional adsorption.

The adsorption of anthrax serum was found to be more complicated because in this case we did not even have an approximate control test which would indicate the end of processing. Agglutination reaction has not been developed and the results of precipitation reaction did not always coincide with the results of the reaction with labeled serum.

For the sorption of the anthrax serum, first we used an equal mixture of different strains of microbes. It was then found that the exhaustion of the group antibodies progressed at different rates: After the labeling of the serum some of the microbes stopped luminescing, and the microbes of other strains continued to exhibit such an intense luminescence that additional adsorption was necessary.

An attempt was made to compare the data of ordinary agglutination and precipitation reactions and the reactions with labeled antibodies for the most heterogeneous adsorbent microbes. For the drop agglutination reaction, we took serum

diluted 1:10 and homologous suspension of the microbes. Antigens for the precipitation reaction were prepared according to the requirements for the standard anthrax antigen. The results of the reaction are shown in the table

| 1) Антиген                                         | 2) Результат реакции      |                        |                                                       |
|----------------------------------------------------|---------------------------|------------------------|-------------------------------------------------------|
|                                                    | 3) капельная агглютинация | 4) преципитация        | 5) опеченение с не-адсорбированной меченой сывороткой |
| Bac. anthracoides № 86 . . . . .                   | ++++                      | 2) +++ через 15 секунд | ++++                                                  |
| Bac. anthracoides № 103 . . . . .                  | +                         | 8) + через 12 минут    | +++                                                   |
| Bac. pseudanthracis № 104 . . . . .                | -                         | 9) -                   | ++++                                                  |
| 6) Стандартный сибирезавесный (контроль) . . . . . |                           | 9) ++++ моментально    |                                                       |

- Legend:
- 1) Antigen.
  - 2) Result of the reaction.
  - 3) Drop agglutination.
  - 4) Precipitation.
  - 5) Luminescence with unadsorbed labeled serum.
  - 6) Standard anthrax (control).
  - 7) In 15 seconds.
  - 8) In 12 minutes.
  - 9) Immediately.

It was found that in the case of separate adsorption of the serum with Bac. anthracoides No. 86 agglutinates were produced in 5 -- 10 minutes, with Bac. anthracoides No. 103 there were fine agglutinates which appeared in 20 -- 25 minutes, while Bac. pseudanthracis No. 104 did not agglutinate at all even in 24 hours. At the same time antibodies which were common to them took the longest time to be eliminated.

Taking into consideration serological peculiarities of the above-mentioned strains, we adsorbed the sera separately with each strain. Microbes were added in a proportion of 1.5 milliard to 1 ml of serum. First we used Bac. anthracoides No. 86 six times with single increasing exposure of 20 -- 25 minutes. Adsorption with microbes Bac. pseudanthracis No. 104 was done in 10 stages with single exposure of 40 minutes. Later, in order to speed up the process, we started processing 5 times with this microbe but doubled its amount.

The adsorption process lasted 1 -- 2 days, after which the serum was centrifuged and filtered through a Seitz filter and through cotton pulp filter. The salting out and the dialysis of globulins, their labeling and further processing were done according to a generally accepted method with some

deviations.

Thus, the adsorption with liver powder was carefully carried out. In general it should not be used for the elimination of free fluorochrome. For this purpose it is better to lengthen the time of dialysis or to reprecipitate the labeled albumins. Liver powder is a very active nonspecific adsorbent which exhausts the serum considerably, and in large doses it is capable of extracting all labeled antibodies in the same way as activated carbon. Paratyphoid sera intended for analyzing preparations from the organs were adsorbed only once with half the amount of liver powder (40 -- 50 mg to 1 ml of serum) in the course of 30 minutes at room temperature. This was done in order to eliminate the antibodies which are capable of nonspecific precipitation on the tissue elements of the preparation. The powder was prepared from the liver of animals of that species to which the studied animals belong. The anthrax serum was not processed with liver powder because it was not intended for the study of pathologic material.

The specificity of the labeled adsorbed serum was checked for the same microbes as the unadsorbed serum. The titer of the serum was predetermined in regard to homologous microbes. The degree of luminescence of the latter was regularly connected with their pathogenicity. For example, avirulent anthrax bacilli\* exhibited a more intensive luminescence than the virulent strains No. 68 and X-3 and not any weaker luminescence than other virulent strains.

Whenever necessary the serum was additionally adsorbed with heterologous microbes which did not yet exhibit luminescence, but this rarely happened. Before the bacterial mass was introduced, the labeled serum was diluted to  $2/3$  of its titer, but not more than 5 -- 7 times. This was done so that the dilution of the serum would not be excessive after processing and it would not be necessary to concentrate it. The degree of residual luminescence of the adsorbent microbes was checked after each instance of centrifuging. The treatment of the serum was stopped when the shell luminescence of the microbes ("capsule") disappeared.

Then the serum was preserved (with merthiolate in concentration of 0.01 % or 1 % of twice recrystallized boric acid), filtered and titrated again. It should be noted that some of the filter plates for the Seitz filters sorbed labeled albumins quite considerably. We did not apply reprecipitation of albumins or other methods of concentrating labeled sera. Adsorption of labeled group sera was carried out in the same way as that of the native ones. They were also diluted not more than 5 -- 7 times. It should be noted that it is more expedient to adsorb the sera before labeling than after it.

The adsorbed labeled sera ensured a weaker luminescence of \*(strain No. 63)

homologous microbes as compared to the unadsorbed ones at identical concentration of the albumin. This was particularly noticeable in the case of the anthrax serum.

In order to intensify the luminescence of the antibodies labeled with fluoresceine isocyanate, we used the data of Bozhevol'nov who had established that this fluorochrome luminesces most intensively at pH= 9.0 -- 12.0. The prepared mounts were alkalized with a carbonate buffer to pH= 9.0 -- 10.0 for  $\frac{1}{2}$  -- 1 minute immediately before the microscopic study, then the solution was decanted and the preparation was dried with filter paper. With this method, the fluorescence was 2 -- 4 times greater.

Labeled adsorbed sera prepared according to the method described above caused a sharp luminescence of homologous microbes (++++) with the following content of labeled albumins: The Gertner serum 0.3 mg, the Breslau serum 0.6 mg and the anthrax serum 0.8 mg per 1 ml. Heterologous microbes exhibited only their own dull contourless luminescence which we evaluated as obviously negative. The checking of the sera done on the conditioned homologous and heterologous strains (Breslau -- 16 and 38, Gertner -- 18 and 38, and anthrax -- 23 and 20 strains).

The adsorption of sera for labeling should be done very carefully because the preservation of the entire set of specific antibodies is more important here than in the ordinary adsorbed sera. Group adsorption is permissible only if similar serological characteristics are present in the adsorbent microbes, in particular when the formation of the agglutinates progresses at the same rate.

A mass formation of agglutinates should not be expected during adsorption. As it has been shown by Gostev and Saakov, specifically agglutinated bacteria eliminate many nonspecific albumins from the solution. From the point of view of the reaction between the adsorbent and the group antibodies, many important strictly specific antibodies may turn out to be such "nonspecific" albumins. The draining of the latter occurred when undiluted serum was adsorbed, substantial doses of adsorbent were introduced, or the exposure was too long.

Adsorbed sera ensure weaker luminescence of homologous microbes, inasmuch as their group antigen determinants cannot be saturated by antibodies. This defect can be considerably corrected if the microscopic study of the preparation is done at a pH which would ensure the most intensive luminescence of fluorochrome.

Labeled precipitating anthrax serum, when adsorbed by the appropriate microbes, permits to differentiate serologically Bac. anthracis from Bac. anthracoides and pseudoanthracis and differentiate between the last two forms. A conclusion can be made from this that the shell of Bac. anthracis

does not have just one somatic antigen but at least three groups of various determinants -- a strictly specific one same as Bac. anthracoides and one identical to Bac. pseudo-anthraxis. Quantitative ratio of these determinants is different in different strains of Bac. anthracis.

Experiments with precipitated anthrax serum have demonstrated a sharply expressed disagreement between the data of the agglutination and precipitation reactions on the one hand, and the luminescent analysis on the other. This disagreement can be explained by the presence of incomplete antibodies in the serum. The use of labeled sera for the study of serological relationship of microbes is obviously of interest, inasmuch as during luminescence microscopy we observe the reactions of complete and incomplete antibodies, while in the agglutination and precipitation reactions only the reactions of complete antibodies can be observed.

#### Conclusions

1. The developed method of a gradual draining of group antibodies with a short exposure of the adsorbent microbes allows to preserve the basic mass of specific antibodies in the serum;

2. The method was found to be quite adequate for the preparation of luminescent adsorbent anthrax serum and paratyphoid sera and can be tested for the adsorption of other sera.

#### Literature

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