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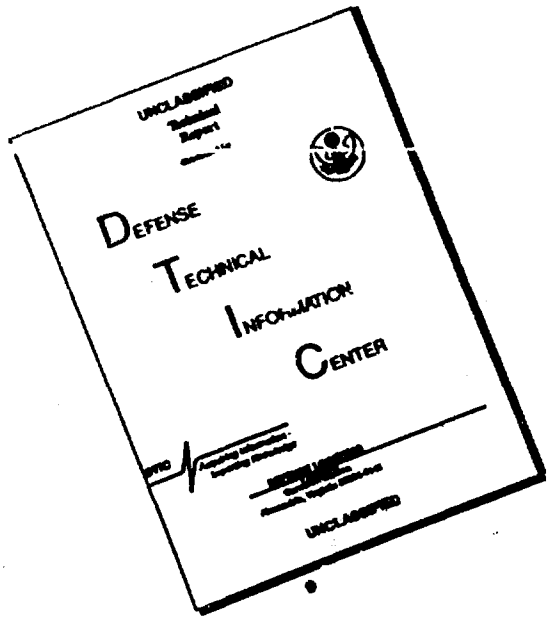

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THE POSSIBILITY OF DETECTING TYPHOID-PARATYPHOID BACTERIA IN THE BLOOD BY USING LUMINESCENT SERA

/Following is the translation of an article by L. V. Mirolyubova in the Russian-language journal Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), Moscow, Vol. 33, No. 3, 1962, pages 14-17./

The luminescent-serological method permitting to detect specific antigenic properties and morphological signs of microorganisms is used more and more in luminescence-microscopic studies. Authors of numerous works have shown the possibility of detecting bacteria of various types in experiments (in pure cultures and in a mixture with other microorganisms) by means of luminescent sera (Moody, Goldman and Thomason, 1956; Hobson, and Mann, 1957; Meisel' with co-authors, 1957; Mikhailov and Li-Li, 1958; Larionov and Kuz'-min, 1959; Dashkevich and co-authors, 1959).

The application of this method for the purpose of diagnosis (for detection and identification of pathogenic bacteria in the discharges of the patients, in the soil and in water) has just begun to develop during recent years (Halpern, Donaldson and Sulkin, 1958; Carter, 1959; Thomason and co-authors, 1959).

Although such works are few, even now it is possible to speak of limited possibilities of the luminescent-serological method in relation to intestinal infections. Since the method of luminescent sera is based on the reaction between the antigen and the antibody, then, due to a broad similarity of the antigens of the bacteria of the intestinal group, it can be applied only for obtaining approximate results (Kabanova, Kuznetsova and others, 1959; Sinitskii and others, 1959; Labrec and others, 1959). In the few instances when the identification of the group of the microorganisms is done only on the basis of serological data (for example, causative agent of colienteritis), the luminescent serolo-

gical method can be used very effectively (Petuely and co-authors, 1958; Whitaker and co-authors, 1958; Kabanova and co-authors, 1959).

One of the methods of increasing the specificity of the luminescent-serological method in relation to the causative agents of the intestinal group is to use sera which are highly specific in relation to individual antigens.

The goal of this work was to determine the possibility of using luminescent antibodies for the detection of typhoid and paratyphoid bacteria in the blood. Here we were guided by the following considerations: Positive results obtained in this case by means of luminescent antibodies would be quite reliable, because during the first days of illness there can be no para-agglutinating strains or cultures with brought-on properties present in the blood. The use of the luminescent-serological method in this case would permit to shorten considerably the period of study as compared to the classical method of separating blood cultures.

The luminescent sera were prepared by combining globulin fractions of agglutinating typhoid and the adsorbed types of paratyphoid A and B sera with the isocyanate of fluorescein. The specificity of the sera was checked on the homologous and heterologous types of bacteria. For example, the specificity of the fluorescent agglutinating typhoid serum was checked on 18 strains B. typhi and on 118 strains of various heterogeneous types of bacteria. In all of the studied strains of typhoid bacteria which were processed with a luminescent typhoid serum, we observed a bright luminescense along the periphery of the cells. Of the 118 strains of other types of bacteria processed with the same serum, only in one strain of the coliform bacterium did we notice a slight specific luminescence (Table 1).

The preparations were made in the following way: Smears on the slides were fixed for 10 minutes in alcohol or in the Karnua liquid and were processed for 15 minutes with flucrescent sera at room temperature in a humid chamber. After that the smears were washed in a 0.15 M of sodium chloride solution and covered with a glass cover. On the average it took about an hour to make the mounts.

The preparations were studied by means of a microscope MBI-3 and an opaque illuminator OI-17 in a blue light falling from above through the lens. The observations were done with immersion lenses -- achromats, apochromats and phase lenses (90 X 1.25 -- 1.3 and 100 X 1.3), various immersion liquids (1.515 -- 1.52) and eyepieces 5X and 7X. A quartz mercury ball lamp of superhigh pressure SVDSh-250-3 was used as a source of light. Glass light filter SZS-7 + SS-4 + SS-8 were placed between the illuminator and the opaque-illuminator, and additional yellow light filter T-2H or ZhS-18 was placed

on the eyepiece.

Table 1

Verification of Specificity of the Luminescent Agglutinating Typhois Serum

	(1) Вид бактерий	(2) Число штаммов	(3) Специфическое свечение	
			(4) выявлено	(5) отсутствовало
6	Брюшнотифозные	18	18	—
7	Паратифозные А	6	—	6
8	Паратифозные В	9	—	9
9	Дизентерийные	23	—	23
10	Кишечные	35	1	34
11	Паракишечные	33	—	33
12	Стафилококки	8	—	8
13	Сарцины	4	—	4

Legend:
1. Type of bacteria.
2. Number of strains.
3. Specific luminescence.
4. Expressed.
5. Absent.
6. Thyphoid.
7. Paratyphoid A.
8. Paratyphoid B.
9. Dysentery.
10. Intestinal.
11. Paraintestinal.
12. Staphylococcus.
13. Sarcina.

In the absence of luminescence, the mounts were scanned with a phase contrast device by means of an illuminator OI-19. A phase contrast condenser KF-1 was mounted on the same microscope. This made it possible to scan the same field of vision without moving the mount in the blue light (to stimulate luminescence), as well as in the passing light -- with phase contrast.

Experiments for the determination of bacteriemia by means of the luminescent-serological method were conducted on experimentally infected rabbit blood. 5 -- 10 typhoid bacteria were introduced to 1 ml of blood in vitro, which corresponded approximately to bacteriemia observed in patients.

It was found impossible to detect bacteria directly in the smears by means of luminescent sera at such a low insemination of the blood. In order to secure the accumulation of microorganisms, the blood (5 -- 10 ml) was introduced into a liquid nutrient medium (in the ratio of 1:10) and incubated for 6 -- 18 hours at 37°.

Of the 18 various media which we tested (bile and sugar broths of various concentrations, various dilutions of meat-peptone broth and the Hottinger broth, distilled water with certain amino acids), the application of a 5%-bile broth was found to be most convenient.

Our studies have shown that when 25 and even 50 cells were introduced in 1 ml of blood, it was possible to detect microorganisms directly in the smear only after an incubation of 12 -- 18 hours only in some of the experiments.

Of the verified methods for concentrating bacteria, the most expedient was the centrifuging of the nutrient medium with bacteria (after incubation) in the course of 10 minutes at 10,000 revolution per minute. In this case, even when an insignificant initial number of bacteria were introduced, positive results were noted in more than half of the tests (Table 2). Against an overall dull background of the mount prepared from the sediment (after centrigufing), we could clearly see rod-shaped bacteria brightly luminescing along the periphery. When 5 to 25 microbes per 1 ml of blood were initially introduced, individual cells were detected in several fields of vision. When 50 microbes per 1 ml were introduced, dozens of cells were detected in the smears in each field of vision. In the latter case, positive results obtained by luminescence tests were always confirmed by bacteriological studies (Table 3).

Thus, the method of detecting bacteriemia which consisted in accumulating typhoid and paratyphoid bacteria (in a liquid nutrient medium) introduced into blood with their subsequent concentration (by centrifuging) and a study of the sediment by means of luminescent sera is not only a more rapid method but also a more sensitive one than the classical method of isolating a blood culture. This can be explained by the fact that by the luminescent-serological method it is possible to detect cells which are not capable of multiplying.

When blood was infected with dysentery bacteria, intestinal and paraintestinal rods, it was possible to see a great number of nonluminescent cells in the smears. The streptococci and the staphylococci which we added to the blood samples were clearly differentiated from the typhoid-paratyphoid bacteria not only by their morphology but also by the absence of luminescence.

Table 2

Results of the Study of the Sensitivity of the Method Depending on the Incubation Periods and a Subsequent Concentration of Bacteria

(2) Срок инкубации при 37° в 5% желчном бульоне (в часах)	(1) Результат исследования при различном исходном числе бактерий на 1 мл крови					
	(3) 5—25			(7) 50		
	(4) Число исследований	из них положительных		(8) Число исследований	из них положительных	
		(5) до концентрирования	(6) после концентрирования		(9) до концентрирования	(10) после концентрирования
(11) Менее 6	36	0	9	18	8	13
» 12	36	10	29	18	11	18
» 18	36	22	36	18	18	18

Legend:
1. Results of the study at various initial quantities of bacteria per 1 ml. of blood
2. Incubation period at 37° in 5% bile broth (in hours)
3. Number of tests.
4. Positive ones.
5. Before concentration.
6. After concentration.
7. Number of tests.
8. Positive ones.
9. Before concentration.
10. After concentration
11. Less than.

Table 3

Comparison of Results of Studies on the Detection of Bacteria by Means of Luminescent Sera and by the Bacteriological Method

(1) Метод исследования	(2) Результат исследования при разном исходном числе бактерий на 1 мл крови			
	5—25		50	
	(3) Число опытов	(4) Из них положительных	(5) Число опытов	(6) Из них положительных
(7) Люминесцентносерологический	36	36	18	18
(8) Бактериологический	36	24	18	18

[See legend following page]

Legend:
1. Method of study
2. Results of study at different initial quantities of bacteria per 1 ml of blood.
3. Number of experiments.
4. Positive ones.
5. Number of experiments
6. Positive ones.
7. Luminescent-serological.
8. Bacteriological.

Conclusions

1. We described a rapid method of detecting typhoid and paratyphoid bacteria in the blood by means of specific luminescent sera.
2. The method was tested with an artificial introduction of typhoid and paratyphoid bacteria into the blood.
3. The studies we conducted have demonstrated a higher sensitivity of the described method than that of the classical bacteriological method of detecting blood cultures.

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