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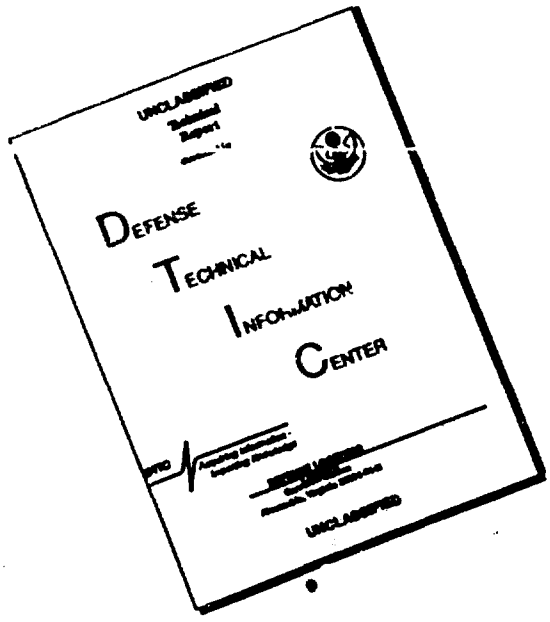

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USE OF THE ANTIBODY NEUTRALIZATION TEST FOR STUDYING THE BODIES OF RODENTS KILLED BY PLAGUE

Mikrobiologiya i Immunologiya Osobo Opasnykh Infektsiy (Microbiology and Immunology of Especially Dangerous Diseases), Saratov, 1964, pp 226-230

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Bacteriological research on rodents and ectoparasites is the basic method for detecting the causative agent of plague in nature. The most valuable material for bacteriological studies on plague, as is well known, are bodies of animals collected in the steppes. On one hand they represent, so-to-speak, a natural selection from the rodent population; on the other hand, the amount of bacteria in theorgans and tissues of the animals killed by infection with plague is much higher than that in the organs and tissues of animals infected, which are caught alive.

However, bacteriological studies of decomposed carcasses of the animals meet with well known difficulties, since isolation of the plague pathogen cannot be successfully accomplished due to the rapid growth of commonplace microorganisms in the culture media. Study of the spinal cord of decomposed bodies of rodents is more effective; however, the causative agent may be successfully isolated for only a short period of time if the carcasses are at a temperature of 20°-30°C.

M. I. Levi and A. G. Momot (1961) developed and laid the foundation for the antibody neutralization test (ANT) in order to identify the specific plague antigen.

This paper is devoted to experimental exploration of several additional improved methods for analysis of rodent carcasses using the ANT, and also for studying the efficiency of this test in connection with the analysis of their individual organs.

Guinea pigs, white rats, and white mice, and meridional gerbils from the left bank of the Volga River, and small susliks (Citellus) were subcutaneously inoculated with virulent strains of the plague pathogen. Carcasses of the fallen animals were kept for 10 days at room temperature (20°-27°C); the ANT was then carried out with a 10% suspension of the decomposed organs. The guinea pigs were studied separately, while the organs from the 10 white mice were collected together in a single test. At the same time agar cultures were made from the same suspensions and also from spinal cord suspensions; white mice were inoculated with these materials.

The suspension of rodent organ or tissue was prepared by grinding in a mortar with sand and washing the pulp which formed with a salt solution. For the ANT 0.25 ml of diluted agglutinating anti-plague serum (two serum units) was mixed with 0.25 ml of a twice-diluted suspension of the spleen, liver, and femural bone; the mixture was left for one hour at 37°C. Then one drop of formaldehyde-treated sheep erythrocytes, sensitized by the 1A fraction of the plague pathogen, was added to each test tube. At the same time all necessary controls were set up. A check of the ANT was made every three hours or on the following morning. In all experiments the same series of agglutinating anti-plague serum (series no. 69, manufactured by the "Mikrob" Institute), and the sensitized erythrocytes, which were agglutinated by the anti-plague serum in the passive hemagglutination test up to a dilution of 1:320,000, were used.

In connection with the participation of heterologous ingredients in the ANT, the sheep erythrocytes and suspension of organs from different kinds of rodents (horse anti-plague serum is not to be considered, since it was introduced in the experiment in very high dilutions), nonspecific clumping of experimental and control erythrocytes was frequently observed, apparently due to heterophyllous antibodies in organ suspensions. Various methods of treating tissue suspensions (mice and guinea pig liver and spleen) were tried in order to remove them: settling or centrifuging by using the upper layer of the liquid, filtering through filter paper after heating for 30 minutes at 56-58°C, with and without agitation of the suspension.

In the majority of cases it appeared that after heating the suspension and with subsequent filtering, the non-specific clumping of erythrocytes was eliminated; other methods of treating the suspension resulted in only partial elimination.

We then tested the effect of similar treatment on a specific titer of the suspension in the ANT of a large number of animals in a somewhat condensed program. Formaldehyde was added to the suspensions for the safety of the experimenters. All variations were tried at the same time in the tests (Table 1).

For comparison the treated suspension was also studied bacteriologically and through bioassay (white mice). In addition, organs of the animal carcasses were cultured by agar impression.

With the latter method a culture was successfully isolated only from the bodies kept at room temperature not longer than 48 hours. Even less frequently, plague pathogens were isolated from the original suspension of organs due to the growth of extraneous microorganisms. Where carcasses were kept longer than 48 hours, isolation of the plague pathogen was possible only by culturing bone marrow of several rodents, and with this method no culture was noted by the end of a week.

No culture was isolated by heating the suspension for 30 minutes at 56°-58°C, nor by heating and treating with a formaldehyde solution (as high as 1% concentration). In administering these suspensions to white mice, the mice did not perish, and no plague causative agent was isolated.

As seen from Table 1, the addition of a concentration of Formalin up to 1% to the suspensions under analysis with subsequent 30-minute heating at 56°-58°C and filtering did not reduce their titer in the ANT. In addition, the treatment as indicated fully sterilized the suspension, thus shortening the experiment.

In a number of cases Formalin and heat treatment of the suspension lead to a rise in the titer in the ANT. A similar phenomenon was noted by M. I. Levi and co-authors (1962) in setting up the passive hemagglutination test. This lead to the selection of treating the suspension with Formalin, heating, and filtering for practical research (qv. "Kratkoye Rukovodstvo..., (Concise Manual...)" 1962).

After working out the methodology, we investigated several dozen carcasses of animals of various types, artificially infected with plague, by means of the ANT (table 2). Only the experiments with the meridional gerbils from the left bank of the Volga River were set up immediately after inoculation of the animals. The suspension from their organs was heated for 30 minutes at 56°-58°C and left standing for 16-18 hours at room temperature. Tested in the ANT was the upper layer of clear liquid.

From Table 2 it is evident that the spleen, as a rule, was the most highly active.

In a special experiment on white mice individual organs which had been kept warm for a long time were tested with the use of the ANT. Mice were sectioned on the day of their death and their spleen, liver, and femural bone extracted and placed in a Petri dish at room temperature for 6-10 days (during this time the organs were becoming dessicated); suspensions of these organs were treated with Formalin, heated, and filtered. In this case a high average harmonic titer of the organ suspension was obtained in the ANT, even after the organs had been preserved for 8-10 days.

TABLE 1

Effect of Various Methods of Treating Suspensions in ANT Titer

(a) Вид животного	(b) Число животных	(c) Штамм	(d) Доза заражения, микробные тела	(e) Время хранения трупа, (сутки)	(f) Суспензия органа	(g) Средняя гармоническая титра суспензий в РНА после различных способов обработки: (h) исходная суспензия	(i) прогретая при 56–58°	(j) прогретая и профильтрованная	(k) формалинизированная, прогретая и профильтрованная
(l) Морская свинка	2	1300	10^4—10^7	0—4	(q) селезенка	1440	1600	3520	3520
					(r) печень	440	800	440	280
					(s) кость	200	400	400	400
(m) Белая крыса	7	"	10^7—10^9	0—6	(q) селезенка	131	69	110	171
					(r) печень	0	0	63	74
					(s) кость	74	64	140	281
(n) Белая мышь	2	.	10	0	(q) селезенка	7680		2560	6400
					(r) печень	5440		2560	7680
					(s) кость	1280			1320
(o) То же	2	1230	10^1–10^9	5	(q) селезенка	960	1600	1280	1600
					(r) печень	21120	21760	12800	21060
					(s) кость	5160	10280	10280	10320
(o) То же	2	1260	10^1—10^9	5—13	(q) селезенка	10880	5440	10560	6400
					(r) печень	1280	1920	1600	2560
					(s) кость	80	80	160	80
(o) То же	1	1252	10	5	(q) селезенка	10240	20480	20480	20480
					(r) печень	640	640	640	640
					(s) кость	320	160	320	320
(p) Малый суслик	6	1300	10^8	0—7	(q) селезенка	19210	16640	16190	16620
					(r) печень	2330	2550	2450	4530
					(s) кость	3960	3850	3890	2330
(o) То же	4	"	10^2	11	(q) селезенка	246080	246080	2	164160
					(r) печень	14240	12960	14240	7200
					(s) кость	1200	1360	1320	1840

Note: The titer is the quantity reversible to the last dilution active in the antibody neutralization test (ANT).

Key:
a--Type of animal
b--Number of animals
c--Strains
d--Infection dose, bacteria
e--Preservation time for carcasses (in days)
f--Organ suspension
g--Average harmonic titer in suspension in ANT after various treatments
h--Original suspension
i--Heated at 56°-58°C
j--Heated and filtered
k--Treated with Formalin, heated, and filtered
l--Guinea pig
m--White rat
n--White mouse
o--Ditto
p--Suslik (Citellus)
q--Spleen
r--Liver
s--Bone

TABLE 2

Activity of Decomposed Organ Suspensions in the Antibody Neutralization Test

(a) Вид животного	(b) Число животных	(c) Штамм	(d) Доза заражения (микробные клетки)	(e) Время хранения трупа (сутки)	(f) Средняя гармоническая титра суспензий органов в РНА: (g) селезенка	(h) печень	(i) кость
j) Морская свинка	4	1300	10^4	2	240	70	
k) Белая крыса	1	"	10^9	0	160	40	160
l) То же	6	"	10^9	7	310	210	910
m) Белая мышь	5	"	10^4	1—3	3580	5630	430
l) То же	1	1230	10^3	5	1280	320	160
l) То же	4	"	10^5—10^6	18	5920	8160	220
l) То же	5	1260	10^3—10^4	15—18	8170	22590	350
n) Малый суслик	1	1300	10^3	2	163840	20480	10240
l) То же	10	"	10^3	15—16	888830	17360	3460
o) Полуденная песчанка	12	1217	10^4—10^6	0	233950	820	4400
l) То же	45	"	10^7—10^9	0	17720	6120	13820

Key:
a--Type of animal
b--Number of animals
c--Strains
d--Infection dose (bacterial cells)
e--Preservation time for carcasses (days)
f--Average harmonic titer of organ suspensions in ANT
g--Spleen
h--Liver
i--Bone
j--Guinea pig
k--White rat
l--Ditto
m--White mouse
n--Suslik (Citellus)
o--Meriones meridianus (gerbil)

In the antibody neutralization test we also investigated the bones (verbebrae) of the midday gerbil (Meriones meridianus) from the left bank. As is known, dessicated bones are often found in epizootic plague foci (Varshavskiy and co-authors, 1957). Titers of suspensions prepared from the vertebrae and preserved for 3-11 days at 20°-28°C were the same as in suspensions prepared on the day of the animal's death.

Conclusions

1. With the use of the antibody neutralization test it is possible to study the decomposed bodies of plague-infected animals. Up to 18 days (the observation period) after the death of the animal, when the carcass is kept at room temperature there appeared to be no regular reduction in antibody activity in the antibody neutralization test (ANT). By using bacteriological or biological methods the plague pathogen was isolated only during the first seven days after the death of the animal.

2. In treating the tissue suspension with Formalin, heating, and filtering the non-specific clumping of erythrocytes was kept to a minimum in the antibody neutralization test, while the activity of the suspension did not change substantially.

3. In analyzing suspensions of the various types of animals perished from plague (132 carcasses) in the antibody neutralization test it was established that the activity of organ suspensions of guinea pigs and white rats was somewhat lower than that of white mice, susliks, and Meriones meridianus gerbils from the left bank of the river. The spleen suspension from the above-mentioned animals often showed the greatest activity.

4. Dessicated soft tissue and bone of the animal's carcass can be analyzed with the antibody neutralization test.

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