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5

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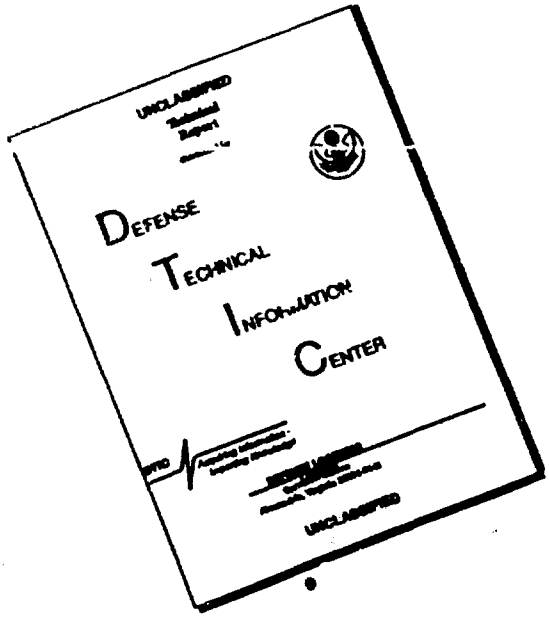

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SEROLOGICAL INVESTIGATIONS ON PLAGUE

Pt. 18. Distribution of Fraction I Antigen in Different Fractions of the Plague Microbe

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The most widely used method of fractionating the plague microbe is that of Baker et al. (1952). Study of the fractions obtained by this method has shown them to be fairly pure. There are considerable losses of fraction I in the course of fractionation.

Immunological analysis of the resultant fractions by means of the reaction of precipitation in gel showed that they as well as the residual liquid contain fraction I antigen.

We set out to determine, using serological methods, how fraction I is distributed in the other fractions obtained by the plague microbe by Baker's method. The object of study was the products of the fractionation of acetone-killed plague bacteria obtained by Baker's method, which has been described in detail by Levi and Momot (1961).

The material under investigation consisted of substances obtained by fractionating 10 strains of plague bacteria. We studied the dry bacterial mass, water-salt extract, fractions 0.2, IA, IB, II and the residual liquid remaining after extraction of fraction II. All the fractions were prepared by M. I. Levi and A. G. Momot in dry form, the drying being carried out after dialysis.

The amount of fraction I antigen in the fractionation products was determined by means of the antibody neutralization reaction (ANR) (Levi and Momot, 1961).

Before titration all the substances investigated were dissolved in physiological solution (pH 7.2) to a concentration of 1000 micrograms in 1 ml, placed in penicillin flasks in an agitator for 1 hour, and left in a refrigerator after formalin was added to a concentration of 1%. The next day the mixture was again agitated for an hour and titrated. The working dilutions of the material prepared in this fashion were stored for several weeks in a refrigerator during which time they retained their neutralizing activity.

Parallel investigations showed that the results of the reaction remained unchanged after titration of the centrifugated-off liquid and non-centrifugated material.

The neutralizing activity of all the fractions of each strain was invariably studied in the same experiment, each of which was repeated several times.

Of decisive importance in the investigations were the factors that ensured standard experimental conditions whereby repeated titration at different times over a period of 2 weeks yielded identical results. These included preservation of the investigated material, the use of immune serum of one series and one working dilution, use of sensitized erythrocytes of a single series or, at least, of identical activity, and use of a single series of physiological solution and the method of fractional titration.

Investigation of different fractions of the plague microbe showed that they all possess neutralizing activity (Table 1). The table lists the absolute weight of the minimum neutralizing dose (MND) of each fraction of all the investigated strains expressed in micrograms. It is noteworthy that the absolute values of the MND of the corresponding fractions of different strains of the plague microbe fluctuate within a relatively narrow range, except strain 1230, which is characterized by a somewhat smaller weight of the MND.

The absolute weight of the MND of fraction 0.2 is expressed in tenths of a microgram; the residual liquid after extraction of fraction II, in tenths and hundredths of a microgram. The MND of the bacterial mass is characterized by values in hundredths of a microgram; water-salt extracts and fractions IA, IB, II, by thousandths of a microgram.

It is interesting to note that the MND for fractions IA and IB of the same strain had the same absolute weight; fraction II, 0.002-0.003 μg higher. A water-salt extract of 5 strains (1256, 1229, 1230, 1260, 1204) had a MND of a larger weight than did fraction I, but it was no more than twice as large. In 4 strains (1254, 1217, 1213, 1252), the MND of a water-salt extract was even less than that of fraction I.

Table 1

Weight of Minimum Nautralizing Dose and Neutralizing Activity in Different Fractions of the Plague Microbe

Штамм (1)	Бактериальная масса (2)	Водно-солевой экстракт (3)	Фракция 0,2 (4)	Фракция I (5) A	Фракция I (5) B	Фракция II (6)	Остаточная жидкость после извлечения фракции II (7)
			(8) МНД фракций, *мкг*				
1255	0,02	0,01	0,3	0,005	0,006	0,008	0,4
1258	0,02	0,004	0,3	0,004	0,004	0,008	0,2
1229	0,03	0,007	0,1	0,003	0,003	0,006	0,06
1230	0,006	0,002	0,04	0,0007	0,0009	0,006	0,05
1252	0,02	0,002	0,2	0,007	—	0,008	0,07
1260	0,02	0,008	0,1	0,003	—	0,005	—
1204	0,03	0,009	0,3	0,003	—	0,01	0,4
1254	0,009	0 004	0,5	0,007*		0,007	0,6
1217	0,02	0,004	—	0,009		0,007	0,08
1213	0,06	0,007	0,9	0,008		0,009	0,09
			(9) Выход МНД фракции I из расчета на 100 *мкг* бактериальной массы				
1256	5000	1894	0,7	582	110	553	23
1258	5000	4500	0,3	475	125	325	7,5
1229	3300	1800	0,7	350	75	416	53
1230	16000	8000	5	1840	220	636	27
1252	5000	5650	0,5	144	—	275	53
1260	5000	1362	1	200	—	400	—
1204	3300	2200	0,3	807	—	320	20
1254	5000	3500	0,4	1200		310	0,5
1217	5000	3500	—	890		300	37
1213	1700	2500	0,3	500		470	—

Note. The * designates a case where fraction I was investigated unseparated.

1 - Strain
2 - Bacterial mass
3 - Water-salt extract
4 - Fraction 0.2
5 - Fraction I
6 - Fraction II
7 - Residual liquid after extraction of fraction II
8 - MND of fractions, μg
9 - Yield of MND of fraction I per 100 μg of bacterial mass

Using data on the quantitative yield of the individual fractions (Levi and Momot, 1961) and determining the MND in gravimetric values by means of the neutralization reaction, one can calculate the number of MND for each fraction obtained by fractionating 100 μg of bacterial mass. The results of the calculations, which reflect the neutralizing activity of the fractionation products, are presented in Table 1. It follows from the table that there is a small number of MND in fraction 0.2 and residual liquid after extraction of fraction II. Fraction IB contains 3-5 times fewer MND than do fractions IA and II while the water-salt extract and bacterial mass have many times more.

Analysis of the distribution of fraction I in the other fractions showed that great losses of neutralizing activity take place in the course of fractionation. Fractions I and II differ insignificantly in their neutralizing activity; fraction IB is much weaker, but all together they possess less neutralizing activity than does the water-salt extract or bacterial mass. On the other hand, the absolute gravimetric values of the MND of the extract and fraction IA are equal or nearly so, despite the fact that fraction IA was obtained by repeated reprecipitation is a purified preparation, whereas the extract contains many other substances besides fraction I. This paradoxical phenomenon may be explained by the fact that fraction I in the extract is in another and lighter form.

Bibliography

M. I. Levi and A. G. Momot, Sbornik nauchnykh rabot Elistinskoy protivochumnoy stantsii (Collection of Scientific Works of the Elistinskaya Antiplague Station), No 2, 1961, pp 111-121.

M. I. Levi and A. G. Momot, ibid., pp 207-214.

E. Baker, H. Sommer, L. Foster, E. Meyer, and K. Meyer, J. Immunology, Vol 68, No 2, 1952, p 131.