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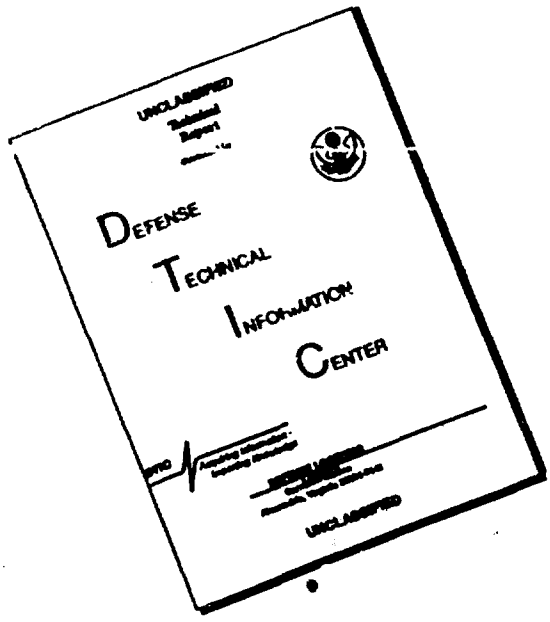

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

INTRACUTANEOUS ALLERGIC REACTION WITH THERMOSTABLE EXTRACTS OF PASTEURELLA PESTIS

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Questions of plague-related allergy have long attracted the attention of investigators. In 1933 N. N. Zhukov-Verezhnikov and T. I. Lipatova first successfully produced the Sanarelli-Shvartsman [latter name transliterated from the Russian; possibly, Schwarzmann] phenomenon on frogs with lysates of P. pestis. This question was studied in greater detail by Z. I. Kolesnikova (1953). V. N. Lobanov (1941) reported that some symptoms observable during plague are characteristic of allergy.

Ye. I. Korobkova (1955, 1956) showed that, in guinea pigs that have had plague and in guinea pigs vaccinated against plague, simultaneously with the development of immunity there occurs an allergic restructuring of the organism, which is manifested in heightened skin sensitivity to a specific allergen -- pestin, suggested by the author, during incutaneous test. The works of the author in this field attracted the attention of other investigators and initiated the search for new preparations to reproduce the intracutaneous test for plague (Zaplatina and Konnova, 1956; Pavlova, 1958; Levi and Shtel'man [both names transliterated from the Russian], 1960; Bakhrakh et al., 1960).

We established (1959) that P. pestis thermostable antigens possess intensely pronounced heteroallergic properties, which are manifested during reproduction of the Sanarelli-Shvartsman [latter name transliterated from the Russian] phenomenon.

Using the intracutaneous test method on guinea pigs, the present work studies the allergenic properties of P. pestis thermostable antigens.

Used in the work were three strains of P. pestis: two virulent -- continental 708 and oceanic 751; and EV vaccine. The method of obtaining thermostable antigens consisted in extracting P. pestis suspensions with physiologic solution by boiling them for an hour.

The microbe suspension containing 40 billion microbes per ml, after being boiled in sealed ampoules, was put in the refrigerator at 5-7° for 1-2 months. During storage the microbes settled to the bottom of the ampoule leaving a transparent supranatant fluid of slightly yellowish color, and this fluid was the subject of the investigation.

Preliminary experiments showed that the thermostable antigens which we had obtained were atoxic and capable of reflecting the allergic restructuring of the organism in immunized and immune guinea pigs. Moreover, the intracutaneous tests made with allergens from virulent or vaccine strains were identically pronounced.

The intracutaneous test was made in accordance with ordinary procedure: a shaven surface of guinea pig body was rubbed with alcohol and the preparation in the volume of 0.1 ml injected; reaction was recorded in 24 hours.

In evaluating guinea pig reaction we distinguished between: sharply positive reaction; positive reaction; slight reaction; and negative reaction.

We considered a reaction sharply positive when there were reddening and infiltrate not less than 2.5 x 2 cm in dimension with marked necrosis or ulcerative blemish of the skin. In the event of positive reaction the infiltrate and reddening attained dimensions of 1.5 x 1 cm with little necrosis or blemish of the skin. Negligible edematousness and limited reddening -- distinguishable, however, in the extent to which pronounced from that of control animals in response to injection of the same antigen -- we rated as slight reaction. In negative reactions skin reddening and swelling were considered probable, but did not exceed the dimensions of the initial papule.

Table 1 shows the effect of thermal treatment of P. pestis on the activity of the allergens obtained.

Table 1

EFFECT OF THERMAL TREATMENT ON ALLERGIC PROPERTIES OF THERMOSTABIE ANTIGENS

1 Антиген	2 Животные	3 Число животных	4 Из них с выраженностью реакции			
			резкоположительная 5	положительная 6	слабая 7	отрицательная 8
9 ТАЕВ-I	14 Подопытные	40	15	20	5	—
	15 Контрольные	21	—	—	1	20
10 ТАЕВ-II	14 Подопытные	10	2	6	2	—
	15 Контрольные	8	—	—	—	8
11 ТАЕВ-III	14 Подопытные	10	5	3	1	1
	15 Контрольные	8	—	—	1	7
12 ТАЕВ-А	14 Подопытные	10	4	4	2	—
	15 Контрольные	8	—	—	1	7
13 ТАЕВ-А-128°	14 Подопытные	5	—	—	—	5
	15 Контрольные	4	—	—	—	4

Keys:

1. Antigen
2. Animals
3. Number of animals
4. Classified by intensity of reaction
5. Sharply positive reaction
6. Positive reaction
7. Slight reaction
8. Negative reaction
9. TAEV-I: thermostable antigen obtained by boiling microbe suspension for one hour
10. TAEV-II: ditto for two hours
11. TAEV-III: ditto for three hours
12. TAEV-A: thermostable antigen obtained by autoclaving at 110° for 30 minutes
13. TAEV-A-128°: thermostable antigen obtained by autoclaving for an hour at 128°
14. Experimental animals
15. Control

Table 2

INTRACUTANEOUS ALLERGIC REACTION PRODUCED BY FILTERED AND UNFILTERED THERMOSTABLE ANTIGENS

1 Антиген		2 Животные	3 Число животных	4 Из них с выраженностью реакции			
				5 резкоположительная	6 положительная	7 слабая	8 отрицательная
9 TAEB сер. 10	ф.[12]	14 Подопытные	л/б[16]	—	14	—	—
	неф.[13]		п/б[17]	11	3	1	—
9 TAEB сер. 10	ф.[12]	15 Контрольные	л/б[16] 10	—	—	—	10
	неф.[13]		п/б[17]	—	—	1	9
10 TAEB сер. 12	ф.[12]	14 Подопытные	л/б[16] 14	3	9	2	—
	неф.[13]		п/б[17]	6	6	2	—
10 TAEB сер. 12	ф.[12]	15 Контрольные	л/б[16] 9	—	—	1	8
	неф.[13]		п/б[17]	—	—	2	7
11 TA708	ф.[12]	14 Подопытные	л/б[16] 7	2	2	2	1
	неф.[13]		п/б[17]	4	2	1	—
11 TA708	ф.[12]	15 Контрольные	л/б[16] 5	—	—	—	5
	неф.[13]		п/б[17]	—	—	—	5

Keys:

1. Antigen
2. Animals
3. Number of animals
4. Classified by intensity of reaction
5. Sharply positive reaction
6. Positive reaction
7. Slight reaction
8. Negative reaction
9. TAEV (thermostable antigen EV) Series 10
10. TAEV (thermostable antigen EV) Series 12
11. TA (thermostable antigen) 708
12. Filtered antigen
13. Unfiltered antigen
14. Experimental animals
15. Control
16. Left side
17. Right side

As can be seen from the Table, the allergen remains active after protracted boiling (2 or 3 hours). Autoclaving at 110° for half an hour does not take allergic properties away from the preparation, and autoclaving for just an hour at 128° inactivates this preparation. Hence, the allergic factors of P. pestis possess high thermostability.

During storage the allergenic properties of the supranatant fluid vary: for the first 3 or 4 months its activity increases, evidently by virtue of the continuing extraction of microbes; upon the expiry of 1.5-2 years it attenuates.

Later on, in order to stabilize the preparation, at the suggestion of A. A. Trifonova we began to filter it through a candle (cherez svechu) and to freeze-dry it in vacuo. Simultaneously the unfiltered supranatant fluid was also dried.

Table 2 shows the results of conducting intracutaneous tests on guinea pigs with filtered and unfiltered dry thermostable antigens obtained from various P. pestis strains. Intracutaneous testing with both (filtered and unfiltered) preparations was performed on one and the same animal.

As can be seen from Table 2, there was practically no discernible decrease in the biological activity of the allergens after filtration and drying; to be sure, in the event that unfiltered antigens were used, sharply positive reactions were noted more frequently.

The preparation preserves its specificity after filtration and drying, as is shown by a special experiment on pigs that had been employed in an immunological check on cholera and plague vaccines. All 19 choleraic pigs on which the intracutaneous test with plague allergen was performed, as well as 5 control pigs, yielded a negative result, whereas the 17 plague test pigs reacted positively to the same allergen.

The results of studying the allergenic properties of the EV strain P. pestis thermostable allergen on guinea pigs enabled it to be tested on people to ascertain the possibility of employing the preparations that had been obtained to reveal the immunological state of the human organism.

We first titrated the thermostable antigen, made from EV (Series 12) vaccine strain, against itself and then tested it on two groups of volunteers. One group of 16 persons was injected with unfiltered dry thermostable antigen, the other group of 9 persons with filtered dry antigen.

Table 3

INTRACUTANEOUS TEST WITH P. PESTIS THERMOSTABLE ANTIGEN ON HUMANS

1 Аллерген	2 Общее количество людей в группе	Вакцинированные систематически 3		Вакцин. давно, или всего 1—2 раза 6		8 Невакцинированные	
		4 резко положительная	5 положительная	5 положительная	7 слабо положительная	слабоположительная	9 отрицательная
10 Термостабильный антиген (нефильтрованный)	16	7	—	3	3	—	3
11 Антиген тот же (фильтрованный)	9	3	1	3	—	1	1

Keys:

1. Allergen
2. Total number of persons in the group
3. Persons systematically vaccinated
4. Sharply positive reaction
5. Positive reaction
6. Persons vaccinated long ago, or once or twice in all
7. Slightly positive reaction
8. Unvaccinated persons
9. Negative reaction
10. Thermostable antigen (unfiltered)
11. The same antigen (filtered)

The preparations were diluted 10-fold with physiologic solution and injected intracutaneously in the central third of the inner surface of the forearm in volume of 0.1 ml.

Each group consisted of three subgroups: the first made up of persons who underwent systematic cutaneous vaccination with live plague vaccine, the second of persons vaccinated once or twice in all, and vaccinated long ago but not vaccinated recently for one reason or another; and the third of five unvaccinated persons, of whom three were injected with unfiltered antigen and two with filtered.

It follows from Table 3 that of the 11 persons who underwent systematic vaccination 10 had a sharply positive reaction in the form of hyperemia and infiltrate in an area not less than 6 x 5 cm, and one a positive reaction with hyperemia and

infiltrate in an area 3 x 3 cm. Of the nine persons vaccinated long ago or vaccinated once or twice in all, 6 had a positive reaction and 3 a slightly positive one (reddening and infiltrate less than 3 cm). Of the five persons never vaccinated, four had a negative reaction, and one a slighly positive one. In the positive cases the reaction markedly attenuates in two or three days, then disappears leaving a slight pigmentation behind.

Thus on a small contingent of persons (25 people) we obtained pretty clearcut results by which the specific activity of the preparation can be judged.

In order for the preparation which we have obtained to be recommended on a wide scale as an allergen to determine immunity amongst vaccinated persons, it must be tested on a large sampling of people.

Conclusions

1. Thermostable antigens intracutaneously injected are capable of reflecting allergic reorganization of the organism in immune, as well as immunized guinea pigs which in this event exhibit positive local inflammatory reaction. Non-immunized guinea pigs react negatively.

2. The intracutaneous test, staged on 25 volunteers, yielded positive results for all persons systematically vaccinated; more less pronounced positive reactions were observed for persons who had received one or two vaccinations. A negative reaction was observed in the case of persons who had not been vaccinated at all, and a slight reaction in persons vaccinated long ago.

3. Filtering the allergen through a candle (~~chorea kaolin~~) and drying it stabilized the preparation with almost no reduction in its activity.

4. No connection was found between the virulence of P. pestis and its allergenic properties.

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