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Some Aspects of Relationship between Antigens of *Pasteurella pestis* and *Pasteurella pseudotuberculosis.* (22816)

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Schütze(1) established that *Pasteurella pseudotuberculosis* and *P. pestis* shared a common somatic antigen. Bhagavan *et al.* (2) have recently confirmed Schütze's finding and have presented data indicating that 5 of 7 *P. pestis* antigens are shared with *P. pseudotuberculosis*. Fraction I of *P. pestis*, according to the authors, is not shared with *P. pseudotuberculosis*. Since the toxin of the plague bacillus is considered an endotoxin, as originally stated by Rowland(3), it can be postulated that as an antigen it would be located in the somatic complex. The study of plague toxin has, in fact, usually been preceded by some operation designed to rupture the cells, such as prolonged growth followed by treatment with toluene(4), alternate freezing and thawing(5,6), and neutral salt extraction of acetone-killed bacilli(7). Somatic antigens of *P. pestis* which are shared with *P. pseudotuberculosis* do not include the plague toxin (2,8,9). For this reason, the toxin of *P. pestis* may be defined as a somatic antigen which is not shared with *P. pseudotuberculosis*.

The present communication describes attempts to establish these criteria using a previously described gel-precipitin technic(10, 11) by comparing lysed and unlysed suspensions of *P. pestis* and lysed suspensions of *P. pestis* and *P. pseudotuberculosis*.

Methods and materials. (a) *Preparation of cell suspensions*: Organisms were grown on heart infusion agar (Difco) at 37°C and harvested in phosphate buffered saline at pH 7.0. Organisms used were *P. pestis* strain A1122 and *P. pseudotuberculosis* strain 1, received from Dr. S. F. Quan, Communicable Diseases Laboratory, USPHS, San Francisco, Calif. The final suspensions contained 1.97-2.00 mg total nitrogen/ml. (b) *Rupture of cells*: Suspensions described above were placed in the glass receiver tubes of the Mickle disintegrator(12). Approximately 5 g of "ballottini"(12) were added to 20-30 ml of organism suspension. The instrument was allowed to vibrate at its maximum amplitude for 15 minutes (time determined by preliminary trials to be described in the next section. (c) *Determination of patterns in 2-channel comparator cells*: The technic used was similar to that described previously(10,11), using 1:50 anti-plague serum globulin in 1% clarified agar as internal reactant(13). *Experi-*

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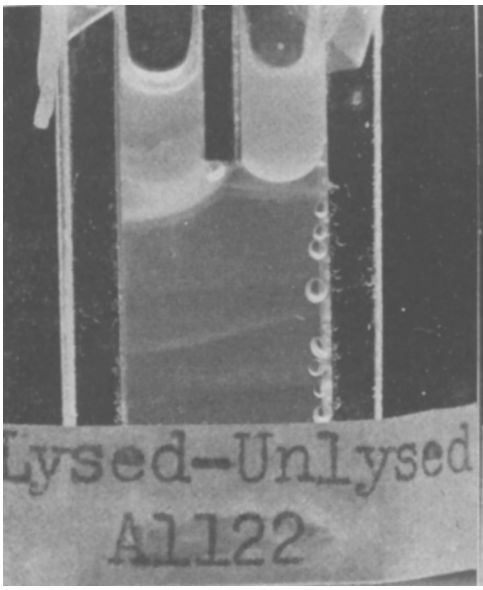

Fig. 1

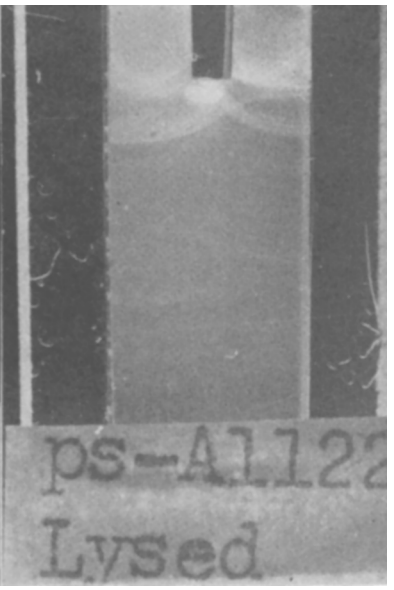

Fig. 2

FIG. 1. Antigen comparator cell showing match between a lysed suspension of *Pasteurella pestis* strain A1122 (on the left) and an unlysed suspension.

FIG. 2. Antigen comparator cell showing a match between lysed suspensions of *P. pseudotuberculosis* (left) and *P. pestis* strain A1122 (right). Approximately 2× enlargement.

mental observation. Three types of experiments were carried out and will be reported in this paper: (1) Lysis of suspensions of plague bacilli; (2) Comparison of lysed and unlysed suspensions with respect to antigenic components; (3) Comparison of lysed suspensions of *P. pestis* with those of *P. pseudotuberculosis*.

(1) *Lysis of suspensions of plague bacilli*: As mentioned above, suspensions of *P. pestis* strain A1122 were subjected to high speed vibration in the Mickle disintegrator in the presence of very small glass beads (ballottini). At 10-minute intervals specimens were withdrawn, the turbidity noted, and added to an aliquot of formalinized saline. Direct smears and cell counts using the Petroff-Hausser counting chamber, were made on the formalinized suspensions. The smears were Gram stained, density of cells and morphological characteristics were evaluated qualitatively. In general, high speed vibration produced with time a diminution in cell count accompanied by a decrease in turbidity of the cell suspensions. Approximately 90% of the reduction in cell count occurred during the first 15-20 minutes of shaking; subsequently, little change took place. The data indicated that this was due to an inherent inefficiency of the process with respect to small coccoid forms. Cultures of plague bacilli are usually mixtures of these and of larger bacillary forms. The disappearance of the latter was readily followed on the Gram stained smears. The action of high frequency vibration was to destroy selectively the larger forms.

(2) *Antigenic comparison of lysed and unlysed suspensions*: The mixture obtained by lysis in the Mickle disintegrator for 15 minutes was matched in 2-channel comparator cells against a suspension of identical composition except that it was not lysed. The test was set up in triplicate. The results of all 3 tests were essentially as shown in Fig. 1. Three rapidly moving components which were common to both preparations produced precipitin zones at considerable distance from points of origin. The fastest and the slowest of these components were present in similar concentration in both lysed and unlysed suspensions, as judged by the distance of their

precipitin zones from the meniscus. Since the distance of diffusion was not increased by lysis, these zones are marked as being of extracellular origin. The third component in this group was of intermediate mobility with respect to the other two, and its distance from the origin was increased on the side of the lysed suspension. The result, as shown in Fig. 1, was a zone of sharp equivalence point characteristics which curved upward from left to right of the comparator cell. The next two zones were common to both suspensions, but significantly increased in concentration in the lysed suspension, as shown by the greater distance of movement on the left side of the cell in Fig. 1. This condition may be explained by postulating 2 endo-antigens which are present in the intercellular fluid due to leakage or autolysis of some cells, but are markedly increased in concentration when all cells are ruptured. Finally, a broad 6th zone proximating the meniscus is present only on the side delivering material from the lysed suspension. This component must be an endo-antigen present in significant concentration only when most of the cells are lysed.

(3) *Antigenic comparison of lysed suspensions of P. pestis and P. pseudotuberculosis*: Lysed suspensions of *P. pestis* strain A1122 and *P. pseudotuberculosis* strain 1 were matched in 2-channel comparator cells as before. After 10 days, the appearance was that shown in Fig. 2. Visual and photographic observations confirmed the fact that the zones produced by the lysed *P. pestis* suspensions were identical to those produced in the preceding test, except for the presence of 3 instead of 2 zones in the intracellular antigen complex. (a) Two extracellular antigens appeared to be shared by both bacterial populations. These are the most rapidly moving zones which were present in lysed and unlysed *P. pestis* suspensions in like concentration. The third extracellular antigen of *P. pestis* was not shared with *P. pseudotuberculosis,* as indicated by the semi-radial pattern arising from the right (*P. pestis*) side of the cell. This precipitation zone had the same characteristics with respect to density as the antigen whose mobility was increased by lysis in the preceding test. (b) The next two intracellular antigens of *P. pestis* were not shared with *P. pseudotuberculosis.* (c) Finally, there was striking evidence that the slowly diffusing meniscus antigen of *P. pestis* is present in greater concentration in *P. pseudotuberculosis.*

Discussion. The finding of 7 antigens of *Pasteurella pestis* in the avirulent strain A1122 confirms the findings of Bhagavan *et al.* (2). However, it must be emphasized that this pattern does not represent a maximum or fixed number of possible components. Many of the variables involved have been mentioned by Oudin(14), Munoz and Becker (16), Jennings(15), and Ransom *et al.*(11).

The following description appears from this work to represent essentially the antigenic structure of *P. pestis* and *P. pseudotuberculosis.* There are at least 2 antigens common to both organisms which diffuse readily from the surface of the cell, which probably correspond to the "envelope" antigens of Schütze (loc. cit.). A third *P. pestis* antigen which diffuses quite readily from suspensions but is increased in concentration when the cells are disintegrated is not shared with *P. pseudotuberculosis.*

The finding of 2 shared "envelope" antigens between these two organisms confirms the observation of Larson *et al.*(17) that cross reacting antigens are present in ether-treated water-soluble preparations of the two organisms. Since Fraction I has been shown to be absent in *P. pseudotuberculosis,* this antigen is probably represented by the third zone of intermediate mobility referred to above.

There are 2 or 3 antigens which are so intimately associated with the soma that their appearance in the extracellular fluid is limited to relatively small quantities unless the cell is broken up. These *P. pestis* antigens are not shared with *P. pseudotuberculosis.*

There is at least one intracellular antigen which is so firmly bound to the soma that it does not appear in these tests at all unless the cell walls are very largely destroyed. This antigen is possessed also by *P. pseudotuberculosis,* and is perhaps identical with the so-

TABLE I. Distribution of Antigens in *Pasteurella pestis* and *P. pseudotuberculosis* as Revealed by the 2-Channel Comparator Cell.

Probable cellular location	Shared	*P. pestis* only	*P. pseudotuberculosis* only
Envelope	2	1*	0
Somatic	0	3†	0
Intracellular	1	0	0

* Fraction I? † Fraction II?

matic antigen of Schütze.

Since toxin is possessed by *P. pestis,* including strain A1122, but not by *P. pseudotuberculosis,* one of the unshared antigens most probably represents the toxin. Plague toxin is known to be firmly bound to the cell. The only likely candidates then, assuming that plague toxin is represented in these tests by one or more precipitation zones, are the two or three somatic antigens which *P. pestis* does not share with *P. pseudotuberculosis.* The hypothetical distribution of antigens between *P. pestis* and *P. pseudotuberculosis* is shown in Table I.

Summary. It has been shown that high speed vibration of cells of *P. pestis* in the presence of "ballottini" is accompanied by disintegration of the bacillary forms with concurrent release of antigenic material. At least one antigen was present in the lysed material which could not be demonstrated when cells were not lysed. 2. *P. pestis* and *P. pseudotuberculosis* appear to share 2 free "envelope" antigens as well as a single somatic antigen. In addition, it has been shown that *P. pestis* possesses a minimum of 1 "envelope" and 3 somatic antigens which are not shared with *P. pseudotuberculosis.* No antigens which could be considered specific for *P. pseudotuberculosis* were revealed.

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