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Evidence for Molecular Heterogeneity of the Specific Antigen (Fraction-1) of *Pasteurella pestis*

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Fraction-I (F-I) protein, the specific antigen of *Pasteurella pestis*, appears to be a series of molecular aggregates of identical subunits, all of which have serological reactivity with specific F-I antiserum.

In attempts to standardize fraction-I (F-1) as the universal antigen for plague serology, it was noted that preparations of F-1 appeared homogeneous by immunodiffusion analysis and during free boundary electrophoresis. However, in disc electrophoresis and gel filtration this same material appeared to be a heterogeneous mixture of molecular sizes ranging from 20,000 to more than 500,000 molecular weight.

Pasteurella pestis strain A-1122 was grown at 37 C on Casman media base (Difco). F-1 was prepared from a saline extract of the acetone killed cells and purified by repeated ammonium sulfate precipitation at 30 to 33% saturation at 4 C by the method of Baker et al. (1). This purified F-1 was examined by the Ouchterlony double diffusion technique (3) using antiserum prepared in rabbits with whole cells of *P. pestis*. Only a single precipitin band was obtained suggesting little, if any, contaminating protein. Likewise, in free boundary electrophoresis a single migrating component was observed also suggesting homogeneity of the F-1 preparation.

Discontinuous electrophoresis in 5% polyacrylamide gels was carried out by the method of Davis (2). No protein was detectable on gels stained with the common Amido Black in 7% acetic acid staining procedure. However, when trichloroacetic acid was used as a fixative, a large number of bands could be visualized with Amido Black, Coomassie Blue, Crocein Scarlet, or other dyes as shown in Fig. 1. The relative mobility (R_m) of each band was calculated, and the logarithms of the R_m were blotted versus the band number in order of their occurrence from the front. The straight-line relationship obtained (Fig. 2) indicates that the bands differ from each other by equal increments of size, charge, or both. Preliminary experiments with gel filtration, sucrose density gradient centrifugation, and ion-exchange chromatography, in addition to the free boundary electrophoresis data, all indicate that the bands differ by a uniform size only.

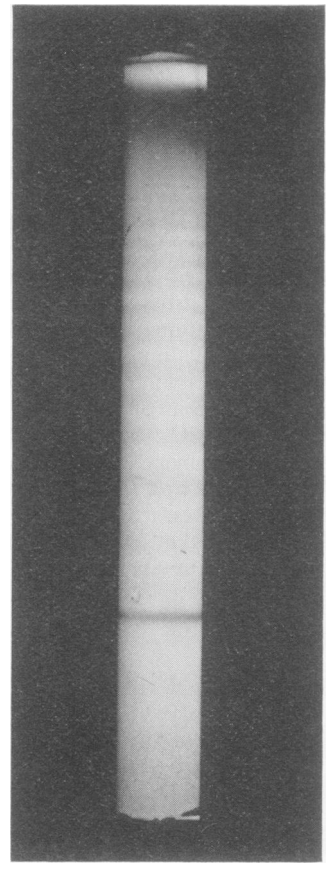

FIG. 1. *Polyacrylamide gel electrophoresis pattern of fraction-I protein. Fixation in 5% trichloroacetic acid containing 0.1% Amido Black.*

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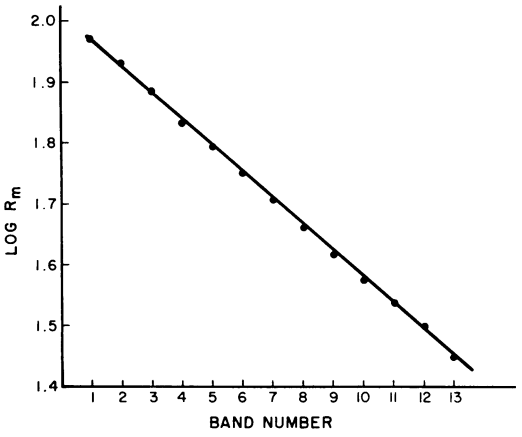

FIG. 2. *Plot of log of relative mobility (R_m) versus band number for fraction-I electrophoresed in 5% polyacrylamide.*

An unstained gel containing protein similar to that shown in Fig. 1 was embedded in agar after electrophoresis. Polyvalent antiplague serum was then placed in a trough parallel to the acrylamide gel. A single precipitin line extended from the origin to the front, attesting to the serological relatedness of the protein components.

At present, work is being directed to identify the factors controlling the size of these polymeric units of F-1 as well as their physical, chemical, and immunological properties.

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