employed as a photographic artifice for bringing out details of internal structure, but by doing so the tails of the phage particles were lost.

FIG. 3. Enlarged and slightly underexposed print of a micrograph showing the W-shaped dense structure in the head portion of individual particles. (50,000 ×.)

FIG. 4. An underexposed print of a micrograph showing a bacterial cell containing rounded but somewhat irregularly shaped bodies suggesting the pentagonal form of the heads of phage particles. Underexposed phage particles of approximately the same size lie in the vicinity of the bacterial cell. (50,000 ×.)

tified in micrographs of one of these (Phage 139). These appeared to be somewhat smaller than those of the phage here described. No distinctive bodies were identified in micrographs of the two other strains (Phages 75 and 177). A remaining strain (Phage 195) was not examined, because of an interruption of these studies.

Summary. A sperm-shaped structure was identified with two bacteriophages active for *Ps. aeruginosa.*

16514

Thermostability and Alcohol Solubility of Vi Antigen.

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The present investigation was suggested by an unpublished observation made by one of us several years ago while working on Vi antigens and antibodies. An antiserum prepared against a motile Vi positive typhoid strain, H-484, was adsorbed 3 times with *Salmonella ballerup.* No drop in titer occurred for either O-901, H-484 or its nonmotile Vi positive variant, O-484. However, a marked prozone, increasing with the number of adsorptions, developed against O-484 (Table I). When *S. ballerup* was washed in several changes of saline before being used for adsorption, the prozone against O-484 failed to appear. It would seem that the Vi antigen was related to this prozone since it was the only component common to O-484 and *S. ballerup.* The present work is an attempt to explain this reaction.

The growth of *S. ballerup* was removed from plates using 5 ml of saline for every 2 plates. The mixture was shaken several times, centrifuged after 30 minutes and the supernatant fluid used to harvest more plates. The procedure was repeated a third time. To 5.85 ml of the final supernatant fluid was added .15 ml of H-484 antiserum. From this mixture 3 sets of serial dilutions were made in saline so that the antiserum dilutions ranged from 40 to 40,960 and the supernatant fluid dilutions from 1 to 1,024. To one set was added .1 ml of a saline suspension of antigen O-901, to another H-484 and to the third O-484. For controls the same antigens were added to saline dilutions of H-484 antiserum. In this experiment the controls are comparable to the tests in Table I labeled "before adsorption" and the mixture of supernatant fluid, antiserum and antigen comparable to those labeled "after third adsorption." Tests were set up in duplicate and after 4 hours at 37°C one test was placed at 2°C for about 18 hours and the other at 55°C for the same time. The results of this experiment, except for small differences in titer and degree of inhibition at the different temperatures, were identical with those shown in Table I.

To test further its ability to inhibit agglutination the supernatant was diluted serially from 1 to 1,024 in saline. To a .5 ml portion of each dilution was added .1 ml of that dilution of pure Vi antiserum just sufficient to give complete agglutination of the test strain in a total volume of .7 ml. The tubes were shaken, allowed to stand about 15 minutes and .1 ml of a saline suspension of O-484

TABLE I.
Adsorption of Typhoid H-484 Vi Antiserum with *S. ballerup.*

Test antigen Before adsorption	Serum dilutions 40	80	160	320	640	1280	2560	5120	10240	20480	40960
O-901	4	4	4	4	4	4	4	4	3	1	—
H-484	4	4	4	4	4	4	4	4	4	3	1
O-484	4	4	4	4	4	4	4	4	3	1	—
After first ads.											
O-901	4	4	4	4	4	4	4	4	2	±	—
H-484	4	4	4	4	4	4	4	4	3	2	1
O-484	1	2	4	4	4	4	4	4	3	1	—
After third ads.											
O-901	4	4	4	4	4	4	4	3	1	—	
H-484	4	4	4	4	4	4	4	3	2	1	—
O-484	—	—	—	1	3	4	4	3	1	—	

4, 3, 2, and 1—degrees of agglutination from complete to weak.

added. As a control antiserum and antigen were added to .5 ml of saline. Tests were incubated at 37°C for 4 hours followed by 18 hours at 2°C. Complete agglutination of the test organism in the control was accompanied by complete inhibition in the lower dilutions of the supernatant. This indicated that free or soluble Vi antigen in the supernatant had adsorbed the Vi antibodies before an effective union could be made with the fixed or cellular Vi antigen. Similar results were obtained with supernatants from the Bhatnagar, Watson Ty 2 and other Vi typhoid cultures using pure Vi antiserum prepared from the first 3 strains.

It was surprising to find almost as good inhibition of agglutination after 18 hours at 55°C as at 37°C in the first experiments since Vi antigen is supposed to be destroyed at 60°C for one hour. To determine the effect of heat on inhibition, saline suspensions of Vi positive organisms were heated to 60°C and in a boiling water bath for from 1 to 3 hours. After centrifugation a clear, fluorescent greenish-yellow "extract" was obtained. These extracts strongly inhibited Vi agglutination, were efficient precipitinogens with pure Vi antiserums and produced strong Vi antibodies upon injection into rabbits. Saline suspensions of O-inagglutinable Vi typhoid strains were heated to 100°C for 1 hour and centrifuged. The extract was positive in tests for the presence of Vi antigen while the washed bacterial bodies were O-agglutinable and failed to agglutinate in pure Vi antiserum. Saline extracts from heavy suspensions of Vi strains heated to 121°C for 3 hours were strongly positive in inhibition tests. These extracts, however, produced only low titering (40 to 80) Vi antiserums after prolonged injection into rabbits which confirms the work of Smith.[1]

All saline extracts prepared from unheated and particularly heated suspensions of Vi positive strains contained high concentrations of O as well as Vi antigen as shown by precipitin tests with O-901 antiserums.

Alcoholic extracts were prepared from *S. ballerup* and Vi typhoid strains by harvesting the growth from plates with absolute ethyl alcohol. The suspensions were placed at 37°C for one or 2 days and shaken frequently. After centrifugation the supernatant was evaporated to dryness at 37°C. The residue was taken up in saline and the insoluble portion removed by centrifugation. To insure the removal of all water soluble material, occasionally the residue was redissolved in absolute ethyl alcohol, centrifuged and the clear supernatant evaporated *in vacuo* over sulphuric acid. These alcohol soluble-saline soluble extracts were strongly positive in all the tests for the presence of Vi antigen, including the production of Vi antibodies.

Saline supernatants, saline heated extracts and alcoholic extracts of a single Vi positive strain varied greatly in their properties, particularly in the concentration of Vi antigen. Saline supernatants showed complete inhibition of Vi agglutination in dilutions of

[1] Smith, E. V. D., *J. Infect. Dis.*, 1938, **63**, 21.

4 to 8, heating from 60°C to 100°C markedly reduced the Vi content and filtering through a Seitz or Berkefeld completely removed it. Saline heated extracts gave complete inhibition in dilutions of 32 to 64, resisted 100°C for 3 hours and produced Vi antiserums with titers as high as 2,560. They could be filtered though some loss in Vi content usually occurred. Alcoholic extracts showed complete inhibition in dilutions as high as 512, were thermostable, have been filtered without loss and have produced pure Vi antiserums with titers as high as 20,480.

Saline supernatants, saline and alcoholic extracts of O-901 and other cultures lacking Vi were uniformly negative in the tests described to detect Vi antigen.

Discussion. The extreme thermolability (destruction or inactivation in 1 hour at 60°C) commonly assigned to the Vi antigen of *S. typhosa* has been questioned by many investigators. Horgan,[2] Robertson and Yu,[3] Smith,[1] Peluffo,[4] Luippold,[5] and others found that the Vi antigen of some strains of typhoid resisted 100°C for varying periods of time up to 1 hour. It is recognized that upon heating the ability of the Vi antigen to (1) cause O-inagglutinability, (2) produce antibodies, (3) agglutinate in antiserum and (4) adsorb antibodies, tends to disappear in the order listed. This progressive loss in characteristics may indicate that the Vi antigen contains more than one component. Obviously the method used to detect the presence of Vi antigen after heating is important and the present report shows that the material used to ascertain the presence of Vi may be more important than the method.

Heating suspensions of Vi strains seems to release all or a major portion of this antigen from the cell. Free in the menstrum the Vi component is relatively thermostable but its disappearance from the cell could easily be interpreted as destruction. The Vi antigen of Felix and Pitt,[6] the alpha antigen of Stamp and Stone,[7] the L antigen of Kauffman[8] and an antigen of *Paracolobactrum intermedium*[9] to be reported later have much in common. They inhibit or interfere with O-agglutination and are more or less widespread in the *Enterobacteriaceae*. Heating releases these antigens from the cell and/or restores O-agglutinability; at least 2 are alcohol soluble.

The relative thermostability and alcohol solubility of the Vi antigen would indicate a possible close relationship to the alcohol soluble heterophile antigens of tissues such as the Forssman, but unlike the latter the former will produce antibodies without the mediation of a conveyor.

[2] Horgan, E. S., *J. Hyg.*, 1936, **36**, 368.

[3] Robertson, R. C., and Yu, H., *J. Path. and Bact.*, 1936, **42**, 53.

[4] Peluffo, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 340.

[5] Luippold, G. F., *Am. J. Pub. Health*, 1946, **36**, 15.

[6] Felix, A., and Pitt, R. M., *J. Path. and Bact.*, 1934, **38**, 409.

[7] Stamp, L., and Stone, D. M., *J. Hyg.*, 1944, **43**, 266.

[8] Kauffmann, F., *J. Immunol.*, 1947, **57**, 71.

[9] *Bergey's Manual*, Sixth Edition, 1948.