tube is 5 cc. The serum and the medium are thoroughly mixed by vigorous shaking. Then 0.1 cc of a 4-day-old culture of *"Donovania granulomatis"* is added to each tube and again mixed well by shaking. These tubes are incubated at 37°C and on the fourth day smears are made from each and stained with Wright's stain and examined under oil immersion lens for the presence of the Donovan micro-organism. The tube containing the least amount of serum that inhibited the growth of the organism is regarded as the endpoint.

Calculation. The least amount of streptomycin that will inhibit the growth of the organism has been found by a similar procedure to be 0.075 γ/cc. Therefore, the serum streptomycin level is equal to 0.075 × Serum Dilution × Total Volume of Medium × 2. For example, if the endpoint of the assay is tube 5, which contains a serum diluted to 1:16, the serum streptomycin level is 0.075 × 16 × 5 × 2 = 12 γ/cc.

With this simple method, a serum level of 0.75 γ/cc to 48 γ/cc can be determined This range embraces the serum levels that are commonly encountered in streptomycin therapy. However, with slight modifications, such as using undiluted serum as the first tube and diluting the serum further, one is able to determine a streptomycin level from 0.375 γ/cc to any level higher than 48 γ/cc.

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Electron Microscopic Observations on *Pseudomonas aeruginosa* Bacteriophage.

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Isolations of bacteriophages active for *Ps. aeruginosa (B. pyocyaneus)* have been reported on a few occasions only. For a time the iridescent erosions which may appear in pyocyaneus colonies were interpreted as manifestations of bacteriophage activity.[1-3] It has now been established, however, that these iridescent changes are unrelated to phage activity and that bacteriophagy among pyocyaneus bacilli is not unlike that observed in other species of bacteria.[4-11] Fastier[12] has recently reported what he erroneously believed to be the first isolation of a pyocyaneus bacteriophage. Between 1930 and 1934 Schultz and Sheets[13] isolated 4 pyocyaneus bacteriophages which are distinguishable from each other and from the one which is the subject of this report.

The present report is based on a strain isolated by one of us (E.W.S.) in 1943 from the exudate of an extensive third degree burn heavily infected with *Ps. aeruginosa*. It bears our number "Phage 238" and is distinguishable from our earlier strains (Phages 75, 139, 177 and 195)[13] by its higher lytic titer and

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1 Hauduroy, P., and Peyre, E., *C. R. Soc. biol.*, 1923, **88**, 688.

2 Hadley, P., *J. Infect. Dis.*, 1924, **34**, 260.

3 Rabinowitz, Genia, *J. Bact.*, 1932, **28**, 237.

4 Quiroga, R., *C. R. Soc. biol.*, 1923, **88**, 363.

5 Combiesco, D., and Magheru, Alice, *C. R. Soc. biol.*, 1923, **88**, 912.

6 Pons, R., *C. R. Soc. biol.*, 1923, **89**, 77.

7 Okuda, S., *Arch. f. Hyg.*, 1923, **92**, 109.

8 Lode, A., *Arch. f. Hyg.*, 1923, **93**, 267.

9 Lisch, H., *Centralbl. f. Bakt.*, I Orig., 1924, **93**, 421.

10 Pesch, K. L., and Sonnenschein, C., *Klin. Wchnschr.*, 1925, **4**, 1585.

11 Asheshob, I., *C. R. Soc. biol.*, 1926, **95**, 1029.

12 Fastier, L. B., *J. Bact.*, 1945, **49**, 633; **50**, 301.

13 Schultz, E. W., and Sheets, Grace, unpublished observations.

wider range of activity for strains of pyocyaneus bacilli. It proved highly active for almost all of 75 "colony strains" cultured from the lesions of this patient over a period of 6 months. There was little tendency on the part of the organisms in the lesions to acquire resistance to the phage, even after its application in liberal amounts as a possible therapeutic measure. It induces complete (4+) lysis of young broth cultures and produces typical plaques on solid media. Secondary growths tend to appear slowly. Filtrates usually titer 10^{-8}. The plaques produced on solid media tend to fall into two groups—large and small, the former measuring about 5 mm in diameter, the latter seldom more than 1 mm. The small plaques appear later than the large ones. Attempts to segregate the components responsible for each of these plaques seem to have been wholly successful. Based upon ultra-filtration end-points with Elford's gradacol membranes, the component responsible for the large plaques appears to be definitely smaller (18-27 mμ) than the component responsible for the small plaques (52-78 mμ). The observations here reported seem to apply only to the larger of the two components (to the "small plaque" phage), the smaller of the two not having been identified in electron micrographs.

Electron microscopic observations on bacteriophages (bacterial viruses) active for certain species of bacteria have already been reported.[14-23] Except for a preliminary report of these observations,[24] none have been reported on the structure of pyocyaneus bacteriophage. A sperm-like form has characterized most of those reported, but differences have also been observed.[25,26] It seems desirable therefore that observations on various bacteriophages be recorded.

Procedure. The lysate was produced in a synthetic medium consisting of asparagin (1%), NaCl (0.5%), $MgSO_4$ (0.2%) and KH_2PO_4 (0.1%) in distilled water, adjusted to pH 6 with NaOH. This medium proved more serviceable than broth. Both the organisms and phage were seeded to the medium in the small amounts customarily employed in setting up such tests, along with the usual controls. The lysed culture was filtered through an L_3 Chamberland candle and 5 cc of the filtrate was then transferred to a chemically clean, sterile centrifuge tube. Enough growth from an 18 to 24 hour agar culture of the homologous organism was then transferred to the filtrate, with a platinum loop, to make it barely cloudy to the naked eye. This mixture was incubated for 5 to 30 minutes at 37°C, then chilled with cracked ice and centrifuged at 2500 r.p.m. for 30 minutes in a refrigerated angle centrifuge at 4°C. The sediment was resuspended in 1 cc of chilled distilled water and again centrifuged. The sediment was now resuspended in 0.5 cc of a chilled 10^{-5} dilution of Zephiran chloride, a surface tension depressant, in distilled water. After slow centrifugation to remove the larger aggregates, the supernatant was transferred to the specimen holder by means of a capillary pipette or platinum loop. The procedure from here on was that ordinarily followed in electron microscopy. A

[14] Ruska, H., *Naturwissenschaft*, 1940, **28**, 45; 1941, **29**, 367.

[15] Pfankuch, E., and Kausche, G. A., *Naturwissenschaft*, 1940, **28**, 46.

[16] Luria, S. E., and Anderson, T. F., *Proc. Nat. Acad. Sci.*, 1942, **28**, 127.

[17] Luria, S. E., Delbrück, M., and Anderson, T. F., *J. Bact.*, 1943, **46**, 57.

[18] Baylor, M. R. B., Severens, J. M., and Clark, G. L., *J. Bact.*, 1944, **47**, 277.

[19] Sharp, D. G., Taylor, A. R., Hook, A. E., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 259.

[20] Edwards, O. F., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 16.

[21] Baylor, Martha B., and Clark, G. L., *J. Bact.*, 1947, **53**, 49.

[22] Lépine, P., Giuntini, J., Croissant, O., and Nicolle, P., *Ann. Inst. Pasteur*, 1947, **73**, 582.

[23] Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 42.

[24] Schultz, E. W., Thomassen, P. R., and Marton, L., *J. App. Physics*, 1944, **15**, 726 (Abstract of paper presented at meeting of the Electron Microscope Society of America, November 16-18, 1944).

[25] Ruska, H., *Ergeb. Hyg., Bakt., Immunitätsforsch. u. exp. Therap.*, 1943, **25**, 437.

[26] Delbruck, M., *Biol. Rev.*, 1946, **21**, 30.

few specimens were prepared by pervaporation of 2 liters of filtrate to 10 cc, ultracentrifuging the latter, resuspending the sediment in 2 cc of distilled water, and then proceeding as outlined above. This yielded many more phages per unit area on the specimen holder but a less clean specimen.

Observations. A considerable number of electron micrographs were taken by means of the Stanford electron microscope, described elsewhere.[27] Observations were carried out at 70 to 80 ekv. Analysis of a number of electron micrographs of Phage 238 show that at least one of the components, probably the larger of the two referred to, is composed of a head and tail portion, these parts being in much the same drum-stick type of relationship as the spore is to the rod in sporulated tetanus bacilli (Fig. 1). The head of this combination appears to be pentagonal in shape and to contain a dense filamentous structure. The average diameter of the head is 76 to 85 mμ, while the dimensions of the tail are about 150 mμ by 15 mμ. The form of the filamentous inner structure in the head portion frequently suggests the form of the letter "W" (Fig. 2 and 3). There are, however, some indications that its true projected shape approaches a distorted figure 8; in other words, that it consists of relatively straight longer portions linked together by sharply curved shorter portions. It appears that it is this relatively thick, dense and unyielding filamentous structure within the substance of the head which gives to it its irregular pentagonal shape. This suggests that the different portions of the filament do not lie wholly within one plane. What appears to be a confirmation of this view was obtained when suitably taken electron micrographs were examined stereoscopically. Several stereomicrographs were taken at ± 13° 53 min. tilting angle by means of a special stage employed in the Stanford electron microscope.[28] From these observations it appears that the dense filament does not lie in a plane, but forms an apparently continuous loop. This loop appears to be bent in a mid plane, forming an obtuse angle with the long axis of the loop. Surrounding and stretched between the segments of this filament there is a less dense material which is apparently continuous with the tail portion. The latter possesses about the same density as the former, and may therefore consist of the same material. The density of the tail portion seems to be approximately that of some bacterial flagella. The tail is almost always seen as a straight or uncurved process and seems always to come off the head portion opposite the middle of one of the longer straight sections of the filament, from where, however, it may come off at different angles.

A few apparently intact bacterial cells were observed which showed rounded internal structures about the size of the heads of the phages here described (Fig. 4). Since some of these appear pentagonally shaped, these may be phages located within the cells. Stereomicrographs served to confirm that these bodies are lying inside of the cell boundary or wall. One of the objectives of these studies was to determine, if possible, the host relationships of the phage and its mode of reproduction. These questions were not answered, however. While many apparently intact bacterial cells were observed with phages on or near their surfaces, also many cells which had apparently undergone phage lysis, little was revealed in the micrographs as to where and how the phage is reproduced.

A considerable number of micrographs of specimens prepared from phage untreated cultures of the organisms employed in these studies failed to show the structures here described as pyocyaneous phage. A highly resistant lysogenic culture, found among the many "colony strains" originally isolated from the patient, was lost before these studies were undertaken and several attempts to create and isolate a similarly resistant strain by artificial exposures to phage proved unsuccessful. It would be of considerable interest to observe the relationships of the phages produced by highly resistant lysogenic strains.

Three other strains of pyocyaneous phages were examined less extensively. A few definitely sperm-shaped structures were iden-

[27] Marton, L., *J. App. Physics*, 1945, **16**, 131.

[28] Marton, L., *J. App. Physics*, 1944, **15**, 726.

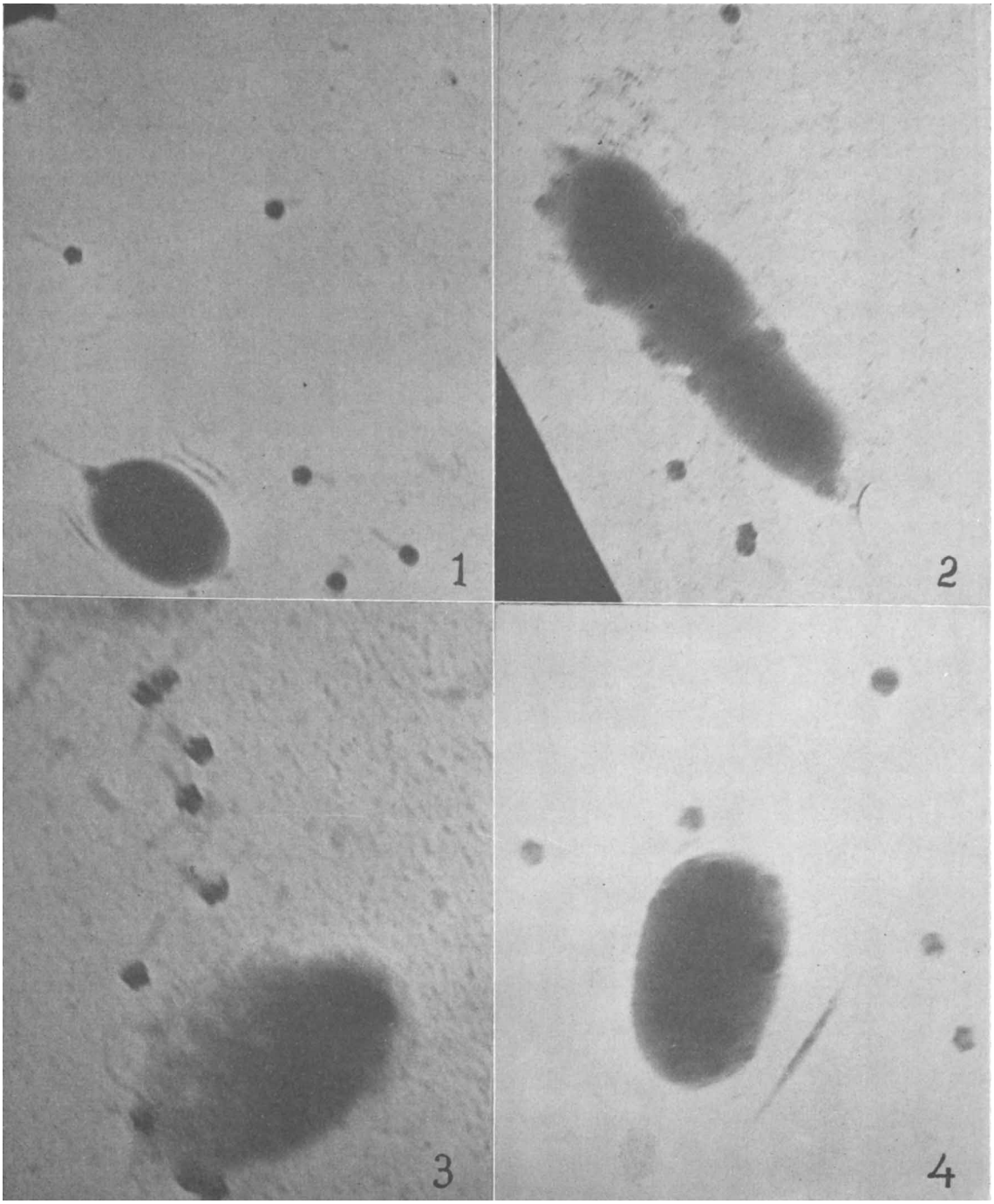

FIG. 1. *Ps. aeruginosa* bacteriophage particles, showing both a head and tail portion. The former is of irregular form due to a rigid internal structure. One of the phage particles is adsorbed to a bacterial cell. (30,000 ×.)

FIG. 2. An underexposed print of a micrograph showing the characteristic W-shaped internal structure in the head portions of the phage particles. Several of the particles are adsorbed to a dividing bacterial cell. (30,000 ×.) The underexposure in making the print was

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.*

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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