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SEROLOGY, DENSITY, AND MORPHOLOGY OF STAPHYLOCOCCAL PHAGES

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ABSTRACT

ROSENBLUM, E. D. (The University of Texas, Dallas), AND SUE TYRONE. Serology, density, and morphology of staphylococcal phages. J. Bacteriol. **88**:1737-1742. 1964.—A correlation between serology, buoyant density, and morphology has been demonstrated for six serological groups of staphylococcal phages. Four morphological types have been observed and represent the following serological groups: (i) group A, (ii) groups B, F, and L, (iii) group D, and (iv) group G. The correlations were useful in the detection of serological variation among several staphylococcal typing phages.

Staphylococcal phages have been grouped by Rountree (1949) and Rippon (1956) by serology and other properties. Seto, Kaesberg, and Wilson (1956) reported differences in morphology between phages of serological groups A and B. The morphology of additional phages was studied by Bradley and Kay (1960), and Bradley (1963) divided the staphylococcal phages into two morphological groups. Phages of serotypes B, F, and L were placed in one group, and serotype A phages in the other group. The former group was subdivided into long tail (group F) and short tail (groups B and L) sections.

In a study of transducing and nontransducing preparations of staphylococcal phages, Dowell and Rosenblum (1962*a*) observed a difference between group A and group B phages in their buoyant density in CsCl. This study was undertaken to determine whether a correlation did exist between serology and buoyant density of the staphylococcal phages, and to determine the morphology of additional phage particles.

MATERIALS AND METHODS

The serological group B phage C has been described previously (Dowell and Rosenblum, 1962*a*). The other phages studied are listed in Table 1.

The serological grouping of the phages (Anderson and Williams, 1956) was not examined further, except for the few phages of unknown serotype, and in instances of anomalous results. These phages were tested for neutralization with antisera for groups A, B, and F (Dowell and Rosenblum, 1962*a*).

Buoyant-density studies. In preliminary studies, the buoyant densities were determined for phage C and typing phage 81 by preparative density-gradient centrifugation in cesium chloride (Dowell and Rosenblum, 1962*b*). In subsequent studies, the phage lysate was centrifuged, and the pellet was resuspended in a small amount of suspension medium (Weigle, Meselson, and Paigen, 1959), mixed with CsCl solution, and adjusted to a final density of 1.480. The mixture was placed in a quartz tube and centrifuged (SW 39L rotor in a Spinco model L preparative ultracentrifuge) at 27,000 rev/min for 18 hr. The position of the phage band was then determined by measuring the ultraviolet (260 mμ) absorption at 1-mm intervals in a Beckman DU spectrophotometer equipped with a vertical scanning attachment. Phage C or 81 was added as a density standard. They were not included when examining lysates that were suspected of containing two components of differing densities.

Electron microscopy. The negative contrast method described by Bradley and Kay (1960) was adapted for the preparation of phages for electron microscopy. A centrifuged phage pellet was suspended in 2% ammonium acetate, mixed with an equal volume of 2% potassium phosphotungstate, and placed on a carbon-coated grid. Electron micrographs were taken with an RCA EMU-3 electron microscope.

RESULTS

Density of reference phages. The buoyant densities of phages C and 81 were determined with gradient centrifugation in CsCl by collecting and assaying dropwise fractions and pooled fractions for phage titer and density, respectively. Three separate determinations for each phage were

TABLE 1. *Serological grouping and sources of phages*

Serological group	Phage strain	Source
A	3A, 3B, 3C, 6, 7, 42B HJD, 42B LH, 42E, 47, 54, 70, 73, 75, 81, 83B(variant), 971	J. E. Blair[a]
	31(variant), 42C (variant), 42F, 47A, 47B, 47C, 51, 57, 75A, 75B, 76 (variant), 78	E. T. Bynoe[b]
B	29, 39, 44A LH, 52 HJD, 52 LH, 52A, 53, 79, 83A, 83B, 523	J. E. Blair
	31, 31B, 42C, 44, 52B, 69, 71, 80	E. T. Bynoe
	83B	M. T. Parker[c]
D	P1, P14, S3K, muscae	American Type Culture Collection[d]
F	42D 77	J. E. Blair
	76	E. T. Bynoe
G	44A HJD	J. E. Blair
	66	M. T. Parker
	68	R. Wahl[e]
L	187	J. E. Blair

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[c] Central Public Health Laboratory, London, England.
[d] Washington, D.C.
[e] Pasteur Institute, Paris, France.

averaged to give a density of 1.459 for phage 81 and 1.502 for phage C. These phages were also included in a number of runs in quartz tubes, and the positions of the band peaks were determined by scanning in the spectrophotometer. The mean value and 1% confidence limits for 15 observations of phage 81 was 15.70 ± .98 mm from the top of the tube; the mean of 17 readings for phage C was 30.44 ± .94 mm. These mean distances were assumed to be equivalent to densities of 1.459 and 1.502, respectively, and within a linear gradient.

Density of staphylococcal phages. The density of each phage was determined one or more times by centrifugation in quartz tubes followed by spectrophotometric scanning. Figure 1 shows a typical result by this method, the density of the phage being calculated from the position of the peak. The initial and final density tails were caused by the meniscus and the curvature of the quartz tube, respectively. Results for all the phages tested are summarized in Table 2. The difference between groups A and B was quite marked and was greater than the variation within each group. Phages of serological group D were intermediate in buoyant density, as was the single representative of group L. Two of the three group F phages were of the same density as group B, and the group G phages were similar in density to group A.

Four typing phages (31, 42C, 76, and 83B), classified as either B or F (Anderson and Williams, 1956), were found to have densities typical

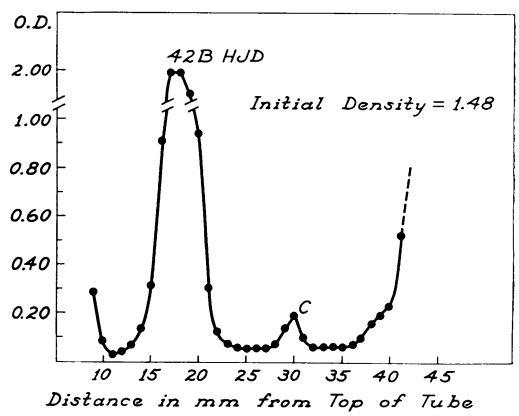


FIG. 1. *Vertical spectrophotometric scan of phages C and 42B HJD after centrifugation in CsCl solution.*

TABLE 2. *Buoyant densities and serological groupings of staphylococcal phages*

Serological group	No. of phages examined	Buoyant density
A	28	1.460 (1.451 to 1.469)*
B	19	1.502 (1.495 to 1.515)*
D	4	1.474, 1.477, 1.480, 1.483
F	3	1.474, 1.498, 1.499
G	3	1.457, 1.457, 1.459
L	1	1.473

* Mean and range for the group.

of group A phages. When tested with antisera, they proved to be group A in serology. Stocks of these four phages of the correct serotype were obtained and examined. The three group B phages were found to have the same densities as other members of group B. The group F phage (76) was less dense than the other two group F phages, but additional group F phages should be examined before this is considered atypical. Thus, the correlation between serology and buoyant density was without known exceptions.

Morphology of staphylococcal phages. The relation between serology and morphology was previously suggested for staphylococcal phages of serological groups A, B, F, and L (Seto et al., 1956; Bradley, 1963). Differences in buoyant density may be related to morphological differences; therefore, representative phages from each serological group were examined under the electron microscope. The results tended to verify the previous findings for groups A, B, F, and L and extended the correlation of serology and morphology to groups D and G. Our results suggested four distinct morphological types of staphylococcal phages (five, when density data is also considered). These are summarized as follows.

(i) *Group A phages.* These phages had distinct oval heads and relatively long tails ending in a terminal knob (Fig. 2, 3). The knob was demonstrated by Bradley (1963) to be a cog-like base plate. Empty heads had a broad band of denser material, and examination of one head, presumably photographed on end (Fig. 3), suggested an inner membraneous structure, similar to that observed by Bradley and Kay (1960) in several phages, including group F and group L staphylococcal phages.

(ii) *Groups B, F, and L.* Phages of these three groups were morphologically similar and had round heads, usually smaller than group A phages, and a relatively short tail ending in a terminal knob (Fig. 4, 5, and 7). The knobs in these electron micrographs were more suggestive of a tail plate than of a spherical organ. Bradley (1963) subdivided the group into short tail (Groups B and L) and long tail (Group F) sections. We did not observe that this distinction was pronounced.

Although the group L phage 187 was similar to phages of groups B and F in morphology, it was distinctly lighter in density and might be placed in a separate subgroup. Bradley and Kay (1960) found that 187 had a slightly wider tail and bigger tail knob than the other staphylococcal phages they examined. This structural difference may account for its lower density. Phage 76, a group F phage with a density similar to phage 187, but different from the other two group F phages, might also be placed in this subgroup.

(iii) *Group D.* This group was distinct from the other staphylococcal phages. It possessed tails with contractile sheaths, cores, and tail plates similar to the T-even coliphages (Fig. 6). These phages were also heterogeneous in head size, a phenomenon observed among other phages by Bradley (1963) and Kay (1963). The Twort bacteriophage (Vieu, Croissant, and Dauguet, 1963), which has a similar morphology, is probably a member of this group.

(iv) *Group G.* This group of small phages were unique among the staphylococcal phages in their apparent lack of a tail, and they possessed a structure analogous to a tail plate which was attached directly to the head (Fig. 8). They resembled pseudomonas phage 12B (Bradley, 1963; Kay, 1963) and the temperate salmonella phage P22 (Anderson, 1960), although they were much smaller (less than 400 A in head diameter) than either of these. Although the serology of phage 44A HJD had not been determined, it was placed in this group, because of its close resemblance to the group G phages 66 and 68 in density and morphology and, like phage 68, its origin as a variant of 44A.

The serological variants of the typing phages mentioned previously were also examined with the electron microscope. The variants, all serological group A, were similar in morphology to group A, and the phages of the correct serotype were morphologically similar to the group B and F phages.

DISCUSSION

Staphylococcal phages of the same serological grouping are quite homogeneous in buoyant density and morphology. The difference in densities between groups is probably due to differences in relative amounts and densities of protein, deoxyribonucleic acid (DNA), and minor components that may be present. Differences in protein:DNA ratios may reflect morphological features such as tail length or size of the head envelope. Variation in the density of the DNA may contribute to the difference in density of the phage particles, but it would take extremes in DNA base composition to produce some of the observed differences. Pre-

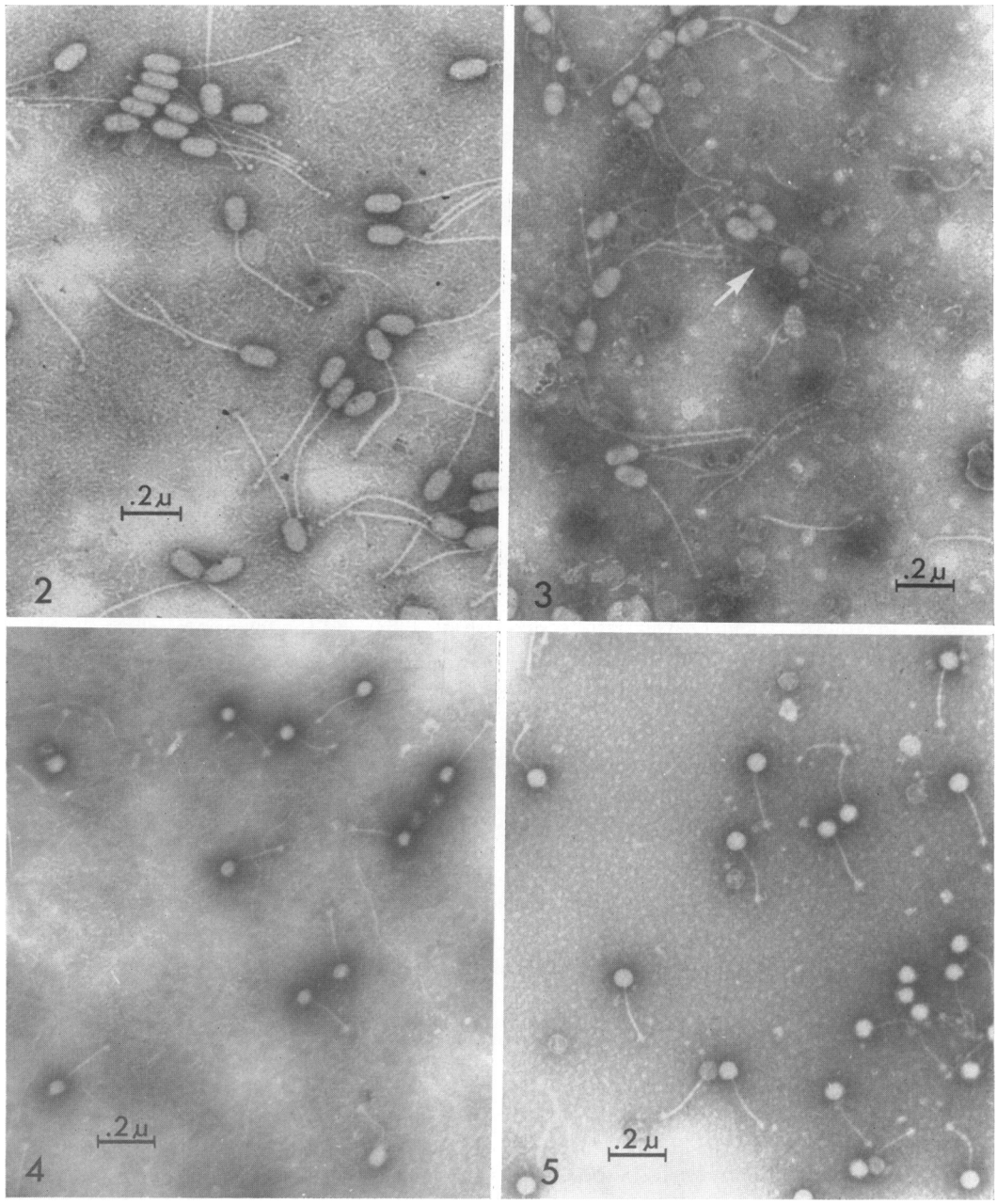
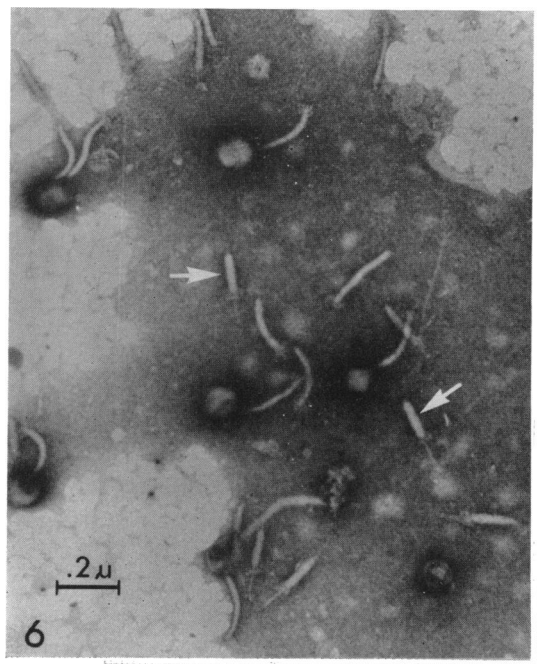
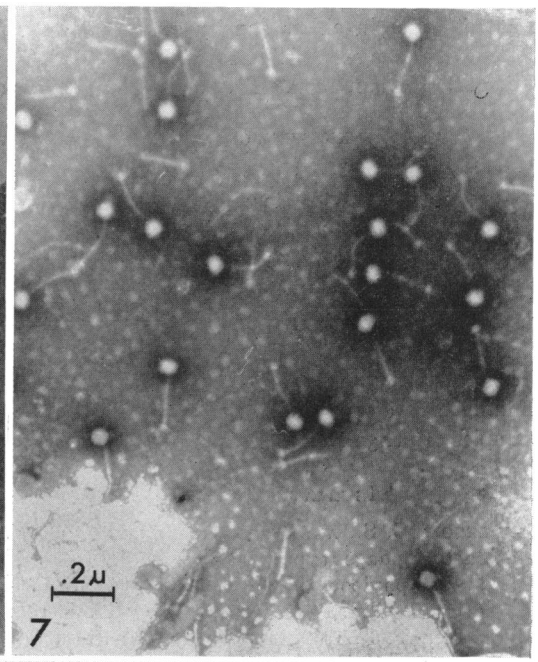


FIG. 2. *Phage 76 (variant) (serological group A).*
FIG. 3. *Phage 42C (variant) (serological group A). Arrow indicates end view of empty head.*
FIG. 4. *Phage 52A (serological group B).*
FIG. 5. *Phage 42D (serological group F).*

liminary studies of DNA from a light group A and a heavy group B phage indicate that they are virtually identical in base composition and density. Further studies of the DNA of representative phages from the other groups may reveal significant differences in composition.

Variation among the staphylococcal typing phages has always been a problem. Both buoyant





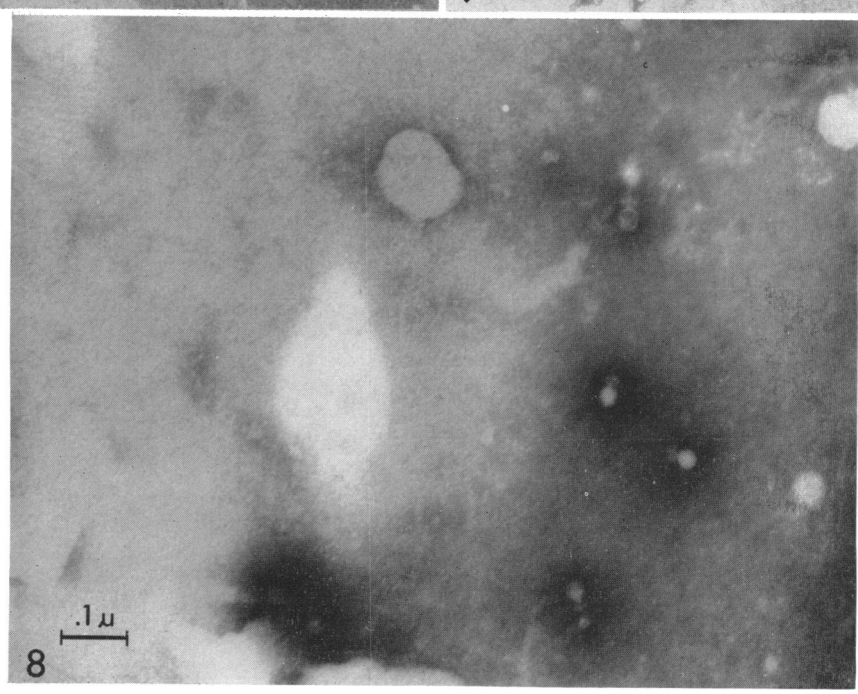


FIG. 6. *Phage S3K (serological group D). Note detached tails with contracted tail sheaths.*
FIG. 7. *Phage 187 (serological group L).*
FIG. 8. *Phage 44A HJD (serological group G).*

density and electron microscopy have been of value in uncovering four instances of serological variation in which phages of groups B or F were replaced by variants of the A serotype. Neither technique would be of value in detecting a change from B to F or vice versa, or in detecting changes in host range alone. Three pairs of such host-range variants were among the phages examined, and

did not differ in either density or morphology. These were phages 52 LH and 52 HJD, 42B LH and 42B HJD, and 83A and 83B.

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