Some Properties of Five New *Salmonella* Bacteriophages¹

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Five bacteriophages were isolated from lysogenic strains of *Salmonella potdam*. On the basis of plaque morphology, thermostability, serology, host range, one-step growth parameters, and phage morphology, they were divided into three groups: group A, phages P4 and P9c; group B, phages P3 and P9a; and group C, phage P10. Group A phages had a hexagonal head 55 nm in diameter with a short tail 15 nm long. These phages were particularly characterized by high thermostability, lack of serological relationship with any of the other phages, and restriction of lysis to other *Salmonella* strains of Kauffmann-White group C₁. Group B phages had a head identical in size and shape to that of the A phages, but they possessed a tail 118 nm long with a contractile sheath. A unique feature was the occurrence of tail fibers at the end of the core rather than at the base of the sheath. These phages were considerably less thermostable, had extended host ranges, and were serologically distinct from each other but unrelated to the A phages. The group C phage, P10, had a head identical to that of the A and B phages. It had a tail 95 nm in length, with tail fibers attached to a base plate at the end of a contractile sheath. P10 was highly sensitive to heat, lysed only smooth strains of *Salmonella*, and showed a degree of serological relationship to both B phages. The relationship of these phage groups to previous *Salmonella* phage grouping schemes is discussed.

Studies of *Salmonella* bacteriophages, particularly those associated with lysogenic strains, appear to have been undertaken first by Burnet (12-14). He described four main subdivisions which he designated as phage groups A, B, C, and D. Later, Boyd published his first paper on the temperate bacteriophages isolated from lysogenic strains of *S. typhimurium* in which he described two main groups of bacteriophages designated subgroup A and subgroup B (8). His subsequent papers were concerned only with the A phages of *S. typhimurium*. The criteria employed by Boyd for assigning an unknown phage to a particular subgroup included plaque morphology, resistance to heat, mutual exclusion, and serology.

With the exception of the *S. typhimurium* A phage P22, and phages that resemble P22, few further systematic studies of *Salmonella* phages appear to have been done (see 10). In view of the great variety of *Salmonella* phages that have been described, this lack of information is a little surprising.

As part of their work on the development of

phage-typing schemes for other *Salmonella* strains, Atkinson and Bullas reported some properties of phages isolated from a number of different *Salmonella* types, including *S. potdam* of the Kauffmann-White group C₁ (3-5). We selected five of these phages for systematic study. This paper presents the results of observations on selected biological properties and on the morphology of these five phages as determined by electron microscopy. Two of the phages have a morphology previously undescribed.

MATERIALS AND METHODS

Bacteria and phages. All *S. potdam* strains were designated by the prefix SP followed by a number—thus, SP2, SP3, SP4, SP9, and SP10. SP2 is non-lysogenic and sensitive to phages released by all of the other strains. The origin of these strains has previously been reported (3). The phages derived from the lysogenic strains were given the prefix P followed by the number of the culture from which they were isolated. Two phages, P9a and P9c, were isolated from SP9. Thus, the five phages used were designated P3, P4, P9a, P9c, and P10. A clear-plaque mutant of P3, P3-c₁, and one of P9a, P9a-c₁, were each derived from spontaneously appearing clear plaques. Phages were isolated by growing the lysogenic strain in mixed culture with SP2 and

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plating out with SP2. The phages were then purified by picking single plaques. The cultures of *Escherichia coli* K and *E. coli* B were obtained from P. D. Skaar, Montana State University, and the culture of *E. coli* C+/L, from D. E. Bradley, University of Edinburgh.

Culture media and methods. L broth and L agar (17) were used for routine growth and plating of both bacteria and bacteriophages. For large-scale preparations, Fraser's medium (15) was used. All cultures were grown with aeration, and all incubations were at 37 C.

Phage assays were performed by means of the standard agar-layer technique described by Adams (1).

Concentration and purification of phage. All five phages were concentrated from crude lysates by differential centrifugation. The crude concentrates of virus were further purified by suspending washed, high-speed pellets in CsCl or in potassium tartrate (P3 and P9a) and centrifuging to equilibrium in a Spinco SW 39 rotor at 35,000 rev/min. The resulting dense bands of phage were collected and freed from CsCl or potassium tartrate by dialysis against phage buffer (7) or 0.11 M ammonium acetate.

One-step growth experiments. The procedure described by Adams (1) was followed.

Sensitivity to heat. Since the different phages showed wide variations in sensitivity to a given temperature, thermostability was described by testing the effect of heat on the number of plaque-forming units over a wide temperature range. Accordingly, 1-ml volumes of phage lysate diluted in broth, or of purified phage diluted in phosphate buffer at pH 7, in small test tubes, were placed in a thermostatically controlled water-bath at selected temperatures for 30 min and then assayed for plaque-forming units. Controls consisted of phage at 25 C. The effect of temperatures between 45 and 90 C on each of the phages was determined in this way. Deviation at a selected temperature was no greater than ± 1 C.

Serology. Antisera against phages were prepared in rabbits. Serological affinities were first determined by a qualitative test. Phages, diluted in broth to a point where they would give countable numbers of plaques when plated out by the standard methods, were mixed with serial twofold dilutions of antisera, made in broth from 1:20 to 1:2,560, and placed at 37 C for 30 min. Controls consisted of the diluted phage mixed with normal rabbit serum collected before immunization. Neutralization was detected when there was a reduced number of plaques in the antiserum-phage mixture compared with the control plates. *K* values of the antisera against those phages which showed serological relationship were then determined by the standard method with the use of selected dilutions of antisera (1).

Host-range lysis. To test for lysis by a certain phage, a loopful of phage lysate was placed in the center of a 1-cm circle or "patch" of the particular bacterial culture to be tested on the surface of an L agar plate. Lysis was observable after overnight incubation.

Electron microscopy. Grids were prepared essentially according to the method of Parks et al. (19). A drop (0.05 ml) of virus suspension in 2.0%

formaldehyde for fixation was placed in the center of each 1-cm agar square and allowed to dialyze dry at room temperature. The agar square was then covered with 0.25% parlodion in amyl acetate, and the excess was drained from one corner of the square. After the parlodion film had dried, the agar square was slowly introduced into a solution of 0.25% uranyl acetate (pH 4.4 to 4.6) or into a solution of 0.75% phosphotungstate (pH 4.7 to 5.8) in a 60-mm plastic petri dish. During this procedure, the film containing virus particles was separated from the agar and floated onto the surface of the stain. The floating film was picked up on a 200-mesh copper grid, dried, and carbon coated.

The grids used in shadowed preparations were prepared differently. Parlodion films were prepared on agar squares before the drop of virus was placed on and allowed to dialyze through. The films were separated from the agar by floating on water, picked up on the grids, and dried. Shadowing was done at a low angle by use of platinum-palladium alloy.

A Siemens Elmiskop 1A electron microscope was used at an accelerating voltage of 100 kv at magnifications of 20,000 to 80,000 times. A decontamination device was occasionally employed.

RESULTS

Plaque morphology. Phages P4 and P9c produced similar, medium-sized temperate plaques with large clear outer rings of lysis. Since these plaques resembled those of Boyd's subgroup A phages and since there were other similarities in the phages as well, these phages also were tentatively classed as group A.

The plaques produced by phages P3 and P9a were similar to one another but different from the group A phage plaques in that the clear outer ring was thinner than the ring in the A plaques. Other properties of these phages were different from the group A phages and, therefore, P3 and P9a were designated tentatively as group B.

The other phage selected, P10, produced a very small, irregular temperate plaque. It was placed in a separate group called group C.

Thermostability. The stability to heat at selected temperatures between 45 and 90 C for each of the phages is shown in Fig. 1. Both P4 and P9c were distinguished by being remarkably stable up to 85 C but were killed at 90 C when heated in broth. A similar degree of thermostability was exhibited by the A phages of Boyd (8, 9). The same phages in buffer showed an increased heat sensitivity but were quite stable up to 70 C and were almost completely sensitive at 80 C.

Phages P3 and P9a were relatively stable in broth to 65 C, were greatly reduced at 70 C, and were completely sensitive at 75 C. In buffer, the number of plaque-forming units was reduced

FIG. 1. Effect of temperature on phage viability. Phage lysates in broth or purified phage in phosphate buffer at pH 7.2 were held for 30 min at various temperatures and assayed for plaque formers. Phage at 25 C was assayed to establish the initial titer. Symbols: ●, P4; ○, P9c; ■, P3; □, P9a; ▲, P10.

even at 45 C. There was a steady reduction in viability up to 60 C and almost complete sensitivity at 65 C.

Phage P10 differed from each of the other phages by showing a similar heat sensitivity pattern in both broth and buffer. Reductions in viability were noted at all temperatures up to 65 C, but a precipitous drop occurred at 70 C. Results of the heat sensitivity tests for all the phages are presented in Fig. 1.

Host range. Both P4 and P9c lysed only *Salmonella* strains that fell into Kauffmann-White group C₁. Since the distinguishing antigen for this group is antigen 7, it is reasonable to suppose that these phages were adsorbed only to cells with this antigen. P3 and P9a lysed a wide range of *Salmonella* strains in many Kauffmann-White groups (5). They failed to lyse *E. coli* B and many freshly isolated strains of *E. coli* obtained from the clinical laboratory of the Loma Linda University Hospital, but they lysed both *E. coli* K and *E. coli* C⁺/L. Growth in either of these two strains of *E. coli* led to a host-induced modification of both phages (L. R. Bullas and R. L. Nutter, *Bacteriol. Proc.*, p. 157, 1969). Both phages were also able to lysogenize *E. coli* K and *E. coli* C⁺/L with high efficiency. About half of the colonies which grew up after plating out the resistant growth from the center of

plaques on these two strains were found to be lysogenic. P10 lysed only smooth strains of *Salmonella* from several of the Kauffmann-White groups from which no specificity for a particular somatic or flagellar antigen could be recognized. No other members of the family *Enterobacteriaceae* were lysed by P10. Thus, the three tentative phage groups were clearly distinguishable on the basis of host range.

Serology. Serological relationships of the five phages are summarized in Table 1. Phages P4 and P9c were very similar serologically and showed no relationship to any of the other phages. Antiserum against P4 had a *K* value of 388 against P4 itself and of 253 against P9c. Antiserum against phage P3 had a *K* value of 72. P3 showed negligible relationship with P9a and none with P10. Antiserum against P10 had a *K* value of 322 and also neutralized to a slight extent both P3 and P9a. P3, P9a, and P10 showed no serological relationship with P4 and P9c.

Thus, P4 and P9c formed a serological group unrelated to any of the other phages. That P3 and P9a were serologically distinct was an interesting result, for they were similar in other ways (e.g., plaque morphology, thermostability, and host range) and, as will be shown, are morphologically identical. Even though P10 showed some serological affinity with both P3

and P9a, it differed from them in other ways and is, as will be seen, morphologically different from each of the other phages.

Cross-immunity. Lysogenic cross-immunity or "mutual exclusion" (8) was shown by phages P4 and P9c which lysed cultures lysogenic with each of the other three phages. On the other hand, cross-immunity was absent between phages P3 and P9a, neither of which conferred immunity to P10. Cultures lysogenic with P10 were lysed by both P3 and P9a. Thus, on the criterion of cross-immunity, P4 and P9c were closely related but P3 and P9a were not closely related; all four of these phages were distinct from phage P10.

Other properties. Each of the phages in lysogenized strains of *Salmonella* was inducible by ultraviolet light and by mitomycin C. Both P4 and P9c were stable to chloroform, but P3, P9a, and P10 were all sensitive. On storage in a refrigerator, lysates of P4, P9c, and P10 were stable over periods of many months, whereas lysates of P3 and P9a very quickly dropped in plaque-forming titer. P4 and P9c could be frozen at -22°C and thawed with little loss in titer, whereas each of the other phages was sensitive to this procedure. P10 was the only phage sensitive to 26.3% urea solution. A summary of these properties of the five phages is given in Table 2.

One-step growth cycles. Minimum latent periods and average burst size values were more markedly different between phages in different groups than between phages tentatively placed

into the same group. As has been previously reported (11), the average burst size of P4 varied from 34 to a high of around 700 plaque-forming units per cell as a function of the age of the phage particles. Aged P4 phage particles yielded larger burst sizes than young phage particles. The minimum latent period was approximately 40 min for all experiments. P9c had an average burst size of about 100 plaque-forming units per cell and a minimum latent period of 40 to 45 min.

The group B phages yielded values of average burst size up to 300 plaque-forming units per cell with minimum latent periods of approximately 30 min. Clear-plaque mutants of each of the phages gave average burst sizes approaching 300 but with shorter minimum latent periods. The latent period for P3-c₁ was 28 min, and for P9a-c₁ was 24 min.

For P10, the average burst size was about 100 with a minimum latent period of 60 to 65 min.

Phage morphology. Bacteriophages P4 and P9c were morphologically similar to each other (Fig. 4). In two dimensions, the heads of each of these phages were regular hexagons 55 nm between opposite points and 48 nm between opposite parallel sides, and were probably icosahedral in shape. The tail, where visible, was approximately 15×9 nm, with a few spikes attached to a base plate apparent. These phages were easy to grow to high titers and relatively easy to purify by differential centrifugation and cesium chloride gradients. Electron micrographs revealed only occasional ghosted phages and very little debris. Since both phages resembled very closely the *S. typhimurium* phage P22 (2, 16) in both size and shape, it is highly probable that they also resemble them with respect to the fine morphological details.

P3 and P9a were very difficult to grow to high titers and to obtain in pure form, even by density gradient methods. In cesium chloride density gradients, there was a loss of viability of up to 99%, and in potassium tartrate density gradients the phage banded close to a band containing high concentrations of flagella. Considerable quantities of bacterial debris were found in close contact with these phages, and in many fields

TABLE 1. Serological relationships of the five bacteriophages in $K \text{ min}^{-1}$

Phages	Antiserum against phage		
	P4	P3	P10
P4	388	0	0
P9c	253	0	0
P3	0	72	7
P9a	0	1	8
P10	0	0	322

TABLE 2. Some additional properties of the five *Salmonella* phages P3, P4, P9a, P9c, and P10

Phage group	Phage	Induction by ultraviolet light and mitomycin C	Sensitivity to chloroform	Stability at 4 C	Stability to freezing and thawing	Urea sensitivity
A	P4	+	—	Stable	Stable	Insensitive
	P9c	+	—	Stable	Stable	Insensitive
B	P3	+	+	Unstable	Unstable	Insensitive
	P9a	+	+	Unstable	Unstable	Insensitive
C	P10	+	+	Stable	Unstable	Sensitive

the phage filaments were found to be attached to these pieces (Fig. 3, 5, and 7).

The morphology of phages P3 and P9a was different from that of P4 and P9c primarily with respect to the length of the tail. Both phages were morphologically identical. The heads of both were hexagonal in shape and identical in size to P4 and P9c. The tail was 118 nm long and 12 nm wide. The majority of phage particles from both cesium chloride and potassium tartrate density gradients appeared with contracted sheaths, even though the heads contained DNA. The contracted sheath was 42 nm long by 16 nm wide; the core diameter was 5.5 nm. In Fig. 2 and 3, P9a particles with sheaths both uncontracted and contracted are shown. Horizontal cross striations are visible in the uncontracted tail sheaths of both phages. The appearance of tail fibers at the ends of the core rather than at the base of the sheath, as in the sheathed, T-even phages, was an unexpected observation. This feature is seen in Fig. 3 and 5.

End to end aggregation of empty tail sheaths and extended tail cores are shown in Fig. 6.

In Fig. 7, a core is shown pulled out from the contracted sheath, leaving it empty. An electron micrograph of a shadowed grid containing a sample of concentrated P3 which had been passed through a cellulose acetate filter (0.45 μ m pore size) is shown in Fig. 8. Phage parts are predominant and include many contracted sheaths standing upright on the grid, as evidenced by their size, shape, density, and length of their shadows. In cross section, the shape of the sheath is hexagonal.

The majority of P3-c₁ and P9a-c₁ phage particles appeared in the uncontracted state, although a few particles were found with filled heads and contracted sheaths. An interesting particle can be seen in Fig. 9. The sheath is split across the middle and, as a consequence, the upper half has contracted while the lower half has remained uncontracted. Thus, sheath contraction occurs in only one direction and would be triggered by relaxation of the mechanism holding the sheath to the base.

Although it is possible that treatments preparatory to electron microscopy were responsible for the sheath contraction, the fact that fibers appeared on the ends of the cores and that the 1 to 2% survivors of the CsCl gradient and the clear-plaque mutants of P3 each showed uncontracted sheaths leaves open the possibility that these B phages were actually infective but were more susceptible to inactivation by CsCl. The tail fibers could then still function in attaching the phage to its host, but the sheath could not function in the process of infection in the same

way as with the T-even phages (21). The extended host range of P3 and P9a might, in some way, be related to this feature of the phage morphology in which the fibers are attached to the end of the core rather than to a base plate which moves with the contracting sheath.

Although electron micrographs were easier to obtain with phage P10 than with either P3 or P9a, many disrupted particles were always seen. The most common form of disruption appeared to be between the head and the tail, yielding ghosted heads and isolated tails (Fig. 13). The survival of P10 in cesium chloride gradients was intermediate between that of P4 and P3.

The general shape of phage P10 was similar to that of P3 and P9a, but a shorter contractile tail, 95 nm long and 12 nm wide, with diagonal cross striations was observed. The head size was the same as that for each of the other phages, with a diameter of 48 nm. Particles with contracted as well as uncontracted sheaths were seen. The contracted sheaths were 38 nm by 17 nm, and the core had a diameter of 6 nm. In Fig. 10 and 11, a structure resembling a base plate, with two parts, one on top of the other, may be seen at the end of the tail, with fibers attached to the distal portion. In contrast to P3 and P9a, the tail fibers are attached to the end of the tail sheath in contracted P10 phages (Fig. 12).

DISCUSSION

The phages that could be isolated from lyso-genic strains of *Salmonella* were first systematically studied by Burnet (12-14). He defined four major groups called A, B, C, and D, distinguished from one another by the criteria of plaque morphology and cross-resistance. Group A phages consisted of those which produced plaques up to 2 mm in diameter with a central mass of secondary growth, frequently surrounded by a halo. Within group A, he defined two subtypes: subtype H (for Hirschfeld or *S. paratyphi* C) since these phages were typically isolated from "Hirschfeld" strains of *Salmonella*, and subtype S (for *suipestifer* or *S. cholerae-suis*) since these phages were typically isolated from "suipestifer" strains of *Salmonella*. The only characteristic that the two subtypes seemed to have in common was their similar plaque morphology. In other properties, they were quite distinct. Thus, A phages of subtype H lysed only smooth strains of *Salmonella*, were heat-stable, and formed a compact serological group unrelated to phages of subtype S. Subtype S phages lysed both smooth and rough strains of *Salmonella*, were heat-labile, and were serologically heterogeneous but unrelated to any of the phages in subtype H.

FIG. 2. Phage *P9a* positively stained with uranyl acetate. The tail sheaths are uncontracted. The bar represents 100 nm.

FIG. 3. Phage *P9a* positively stained with uranyl acetate. The tail sheaths are contracted, and tail fibers are present on the ends of the tail cores connecting the phage to debris (arrow). Same magnification as Fig. 2.

FIG. 4. Phage *P9c* negatively stained with potassium phosphotungstate. The bar represents 10 nm.

The group B and the group C phages of Burnet both produced small, irregular-shaped plaques, but could be differentiated on the basis of "cross-resistance." They were serologically hetero-

geneous. Group D phages were unique in that they produced "very large plaques" up to 8 mm in diameter and appeared to lyse only R forms of *Salmonella*. The *S. typhimurium* phages later

FIG. 5. Phage P3 negatively stained with potassium phosphotungstate. Tail fibers appear on the tail core of one phage (arrow). The bar represents 100 nm.

FIG. 6. Phage P3 parts negatively stained with potassium phosphotungstate. Some tail sheaths are aggregated end-to-end. The bar represents 100 nm.

FIG. 7. Phage P3 negatively stained with potassium phosphotungstate. One contracted sheath has slipped almost off its core. The bar represents 100 nm.

FIG. 8. Phage P3 lysate after passing through sterilizing filter, shadowed with platinum-palladium. Several contracted sheaths are standing on end. The bar represents 100 nm.

FIG. 9. Phage P3-c₁ negatively stained with potassium phosphotungstate. Very fine tail fibers are visible on one phage tail, as indicated by the arrow. The bar represents 100 nm.

described and studied by Boyd (8, 9) were members of the Burnet A and B groups.

The properties of phages P4 and P9c, which we tentatively placed into group A, would place both of them into Burnet's group A subtype H. Thus, both phages produced plaques up to 2 mm in diameter with a central disc of secondary, bacterial growth. They were serologically very similar, were remarkably heat resistant, and lysed only smooth strains of *Salmonella* of the Kauffmann-White group C₁. Morphologically, they were identical and resembled the well known *S. typhimurium* A phage, P22 (2, 16). Other *S. typhimurium* phages that resemble P22 in biological properties have been shown to have a similar morphology (6). Phages similar with respect to type of plaque, thermostability, and host range have been isolated from other strains of *Salmonella*, including *S. bovis-morbificans*, *S. blegdam*, *S. muenchen*, and *S. enteritidis* (5). The epsilon 34 phage of *S. anatum* survives 30 min at 80 C (le Minor, *personal communication*). We have found that the morphology of the phage from *S. blegdam* also resembles that of P4 and P9c. Thus, similar phages are probably widespread among the salmonellae. The classification of P4 and P9c as A phages is justified,

and evidence would suggest that all *Salmonella* A phages may have a similar morphology. Phages P4 and P9c were also serologically similar to group A phages isolated from other Kauffmann-White group C₁ *Salmonella* strains, but were serologically unrelated to the *S. typhimurium* phage, P22.

Phages P3 and P9a possessed many characteristics which would place them into Burnet's group A subtype S: they produced the typical, temperate plaque, lysed both S and R strains of *Salmonella* and other members of the *Enterobacteriaceae*, and, although they were serologically dissimilar to each other, were serologically unrelated to either P4 or P9c.

We have failed to recognize similar phages among those of *S. typhimurium* described by Boyd (8, 9). Two phages which resembled P3 and P9a have, however, been described by Atkinson and Bullas (5). The phages they described were isolated from lysogenic strains of *S. muenchen* and *S. derby*, and we have isolated similar phages from other strains of *S. potsdam*. Burnet's phages of this type were isolated from lysogenic strains of *S. cholerae-suis* (14). Morphologically, both P3 and P9a were identical and were unlike either P4 or P9c, or any of the

FIG. 10. Phage P10 negatively stained with potassium phosphotungstate. A structure with two parts appears at the end of the uncontracted sheath (arrow). The bar represents 100 nm.

FIG. 11. Phage P10 positively stained with uranyl acetate. A structure similar to that in Fig. 10 is visible (arrow). Same magnification as Fig. 10.

FIG. 12. Phage P10 negatively stained with potassium phosphotungstate. Tail fibers are attached to base plates at ends of contracted sheaths. The bar represents 100 nm.

FIG. 13. Phage P10 negatively stained with potassium phosphotungstate. Several empty heads are visible in this field. The bar represents 100 nm.

Salmonella phages cited by Bradley (10). Since the majority of these phages had contracted sheaths, the presence of fibers at the end of the core rather than at the base of the sheath, as in the T-even phages, could be clearly seen and appears to be a unique property. Thus, P3 and P9a represent an entirely new, distinct morphological group of *Salmonella* phages, which we have

called the B group. These phages may be particularly characteristic of group C *Salmonella* strains, although not exclusively because *S. derby* is a member of group B. Phage P10 had a morphology different from that of each of the other phages, and more closely resembled the T-even phages of *E. coli*. Since the tail fibers were found to be attached

TABLE 3. *S. potsdam* phage groups compared with the *Salmonella* phage groups of Burnet (14) and the *S. typhimurium* phage groups of Boyd (8)

Phages	Group	Main criteria	Equivalent Burnet group	Equivalent Boyd group	Criteria of Burnet or Boyd
P4 and P9c	Group A	Plaque morphology Thermostability Host range Serology Morphology Cross-immunity	Type A subtype H	Subgroup A	Plaque morphology Thermostability Host range Serology Mutually exclusive
P3 and P9a	Group B	Plaque morphology Thermosensitivity Host range Serology Morphology	Type A subtype S	Not described	Plaque morphology Thermosensitivity Host range Serology Cross-resistance
P10	Group C	Plaque morphology Thermosensitivity Host range Serology Morphology	Type B or type C	Subgroup B	Plaque morphology Host range Serology Cross-resistance

FIG. 14. A, B, and C types of *S. potsdam* phage morphology. The length and number of fibers per phage particle shown do not indicate the actual state but are diagrammatic only.

to base plates at the ends of the sheaths, the process of infection with P10 may resemble that of the T-even phages, with the insertion of the core through the cell wall (21). Phage P10 was serologically unrelated to either P4 or P9c of group A, but antiserum against P10 showed slight neutralization of P3 and P9a. The long minimum latent period was probably responsible for the very small plaque size produced by P10. Results justified placing P10 into a separate group which we have called group C.

The heads of all five phages were hexagonal in two dimensions and of the same size—55 nm between opposite points. Whether there is any significance to this similarity in size is only conjecture, but it is possible that protein subunits of a given size are made in a variety of phage-infected bacteria. In addition to these five phages of *S. potsdam*, coliphages T3 and T7, P22 and other *S. typhimurium* phages (6), and *Staphylococcus* phages of serological group F (10) also have heads of the same size and shape. Electron microscopy of additional temperate *Salmonella* phages may, in the future, reveal other morphologies than those described here and be the basis of further subdivisions.

A summary of the properties of the five phages of *S. potsdam*, relating them to the previous groups of Burnet and of Boyd, is shown in Table 3. In Fig. 14, profile line drawings of phages representative of groups A, B, and C are shown.

It should be stressed that this phage grouping has reference only to temperate phages that are derived from lysogenic strains of a known *Salmonella* type. Phages such as ϕ X-174 (20), ϕ R, ϕ 2, and 66t (10), which are isolated from other sources and which will also lyse strains of *Salmonella*, are not included in this type of scheme. That a virus may lyse a particular host has, in the past, been the basis of virus classification schemes, but, as Lwoff and Tournier pointed out (18), the ability of many viruses to attack many hosts makes such a criterion unsatisfactory. However, since some temperate bacteriophages such as P3 and P9a are able not only to lyse but also to lysogenize bacteria in another genus as well, a phage classification scheme based on their origin from lysogenic strains is also not without hazard. It is probable that lysogenization of bacteria in one genus with appropriate temperate bacteriophages derived from lysogenic bacteria in a second genus may occur in nature.

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LITERATURE CITED

- Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
- Anderson, T. F. 1960. On the fine structures of the temperate phages P1, P2 and P22. Proc. European Regional Conf. on Electron Microscopy, Delft, Holland, vol. 2, p. 1008-1011.
- Atkinson, N., and L. R. Bullas. 1956. Salmonella bacteriophages. V. Some bacteriophages of *S. potsdam*. Aust. J. Exp. Biol. Med. Sci. 34:455-460.
- Atkinson, N., and L. R. Bullas. 1956. Lysogenicity and lysis patterns in the Salmonellas. X. *S. potsdam*. Aust. J. Exp. Biol. Med. Sci. 34:461-470.
- Atkinson, N., and L. R. Bullas. 1957. Salmonella bacteriophages. VI. Some heat resistant phages. Aust. J. Exp. Biol. Med. Sci. 35:193-208.
- Bezdek, M., and P. Amati. 1967. Properties of P22 and a related *Salmonella typhimurium* phage. I. General features and host specificity. Virology 31:272-278.
- Bowen, G. 1953. Kinetic studies of photoreactivation of bacteriophage T2 inactivated by UV light. Ann. Inst. Pasteur (Paris) 84:218-221.
- Boyd, J. S. K. 1950. The symbiotic bacteriophages of *Salmonella typhimurium*. J. Pathol. Bacteriol. 62:501-517.
- Boyd, J. S. K. 1954. The type-A phages of *Salmonella typhimurium*: two new types. J. Pathol. Bacteriol. 68:311-314.
- Bradley, D. E. 1967. Ultrastructure of bacteriophages and bacteriocins. Bacteriol. Rev. 31:230-314.
- Bullas, L. R., A. J. Zuccarelli, and R. L. Nutter. 1967. Ageing effect on phage particles leading to an increase in burst size. Nature (London) 216:1308.
- Burnet, F. M. 1929. "Smooth-rough" variation in bacteria and its relation to bacteriophage. J. Pathol. Bacteriol. 32:15-42.
- Burnet, F. M. 1930. Bacteriophage activity and the antigenic structure of bacteria. J. Pathol. Bacteriol. 33:647-664.
- Burnet, F. M. 1932. Lysogenicity as a normal function of certain *Salmonella* strains. J. Pathol. Bacteriol. 35:851-863.
- Fraser, D., and E. A. Jerrel. 1953. Amino acid composition of T3 bacteriophage. J. Biol. Chem. 205:291-295.
- Israel, J. V., T. F. Anderson, and M. Levine. 1967. *In vitro* morphogenesis of phage P22 from heads and base-plate parts. Proc. Nat. Acad. Sci. U.S.A. 57:284-291.
- Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1:190-205.
- Lwoff, A., and P. Tournier. 1966. The classification of viruses. Annu. Rev. Microbiol. 20:45-74.
- Parks, W. P., J. L. Melnick, R. Rongey, and H. D. Mayor. 1967. Physical assay and growth cycle studies of a defective adeno-satellite virus. J. Virol. 1:171-180.
- Sertic, V., and N. Boulgakov. 1935. Le grouping des bacteriophages après leur type antigenique. C. R. Soc. Biol. Paris 119:983-985.
- Simon, L. D., and T. F. Anderson. 1967. The infection of *Escherichia coli* by T2 and T4 bacteriophages as seen in the electron microscope. I. Attachment and penetration. Virology 32:279-297.