limiting up to a concentration of 30 to 60 mM. The shape of the curve is such that the exact point is not easily determined. The fact that the ESC-Na content of the bladder wall is lowered by mucosal concentrations of 5 and 15 mM, but not by concentration of 40 mM, is again an argument in favor of the theory that ESC-Na plays a part in the transport process.

The finding of much larger quantities of ESC-Na in bladder wall than in serosal tissue again favors the theory that ESC-Na is a molecular substance involved in the process of transporting sodium against an electrochemical gradient.

Summary. There is in the urinary bladder of *Bufo marinus* an ether soluble compound which binds sodium (ESC-Na). The theory that this compound is in some manner involved in the transport of sodium across the wall of the bladder is supported by the following observations: 1. The content of ESC-Na in the bladder wall is 9.35 μeq/g, while that of serosal tissue, which does not transport Na, is 0.33 μeq/g. 2. The quantity of ESC-Na is reduced by placing a sodium-free solution at either the serosal or mucosal surface. The significance of these findings is discussed. 3. There is a similarity between the effect of increasing the sodium concentration of the mucosal fluid on reduction of ESC-Na concentrations and on rate of sodium transport. 4. The possibility that ESC-Na did not exist as such in the body has been considered. Instead it might be formed after death of the bladder and/or during the extraction process. Data are presented which make this latter possibility unlikely. 5. It is concluded that the data presented support the hypothesis that ESC-Na is a substance which plays a part in the sodium transport process.

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Properties of Staphylococcus Phage UC-18: A Comparison with the International Phage Series. (31646)

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Numerous investigators(1,2,3,4) have reported the experimental use of staphylococcus phages in an attempt to reduce the number of "untypable" cultures. Some of these phages have proved useful in typing cultures which generally show inhibition patterns with Group III phages of the international basic series. One phage (UC-18) has been especially valuable in characterizing cultures from hospital-acquired infections and it has been suggested that this phage be added to the basic series (5). A study of the properties of this phage would be useful before addition to the basic series is proposed formally. This is a report of the properties (morphology, serology, thermal inactivation, buoyant density and burst size) of phage UC-18. A comparison is made on the basis of these properties between phage UC-18 and the phages comprising the basic series.

Materials and methods. Phage UC-18 was furnished by Dr. P. B. Smith (Communicable Disease Center, Atlanta, Ga.). Trypticase soy agar and broth were used for propagation and experimental procedures.

Density gradient centrifugation in cesium

chloride(6) was used to determine the buoyant density of phage UC-18. Experiments were conducted using phage preparations of 10^{10} PFU/ml and banding of the phage was carried out in a Spinco Model L centrifuge (SW 39 rotor) for 17 hours at 22,500 rev/min. Fractions of 10 drops each were titered by the agar layer method and density was correlated to the fraction having maximum infectivity. Cesium chloride densities were calculated as described by Ifft *et al*(7), from refractive indices determined with an Abbe-3L refractometer (Bausch and Lomb). For electron microscopy the banded phage was removed as a single fraction, dialyzed against 2% ammonium acetate, and negatively stained with 2% phosphotungstic acid solution (pH 7.2). Electron micrographs were taken with an RCA EMU-3G electron microscope.

Phage antisera for UC-18 and serological groups F, G, and L were prepared in rabbits. Each animal received a 5 ml subcutaneous injection containing a broth suspension of 10^8-10^9 PFU/ml 15% (v/v) complete Freund Adjuvant (Difco). Injections were given at 5-day intervals for 4 weeks for preparation of grouping antisera and for 6 weeks for preparation of UC-18 antiserum. Group A and B antisera were obtained from Dr. E. D. Rosenblum (Southwestern Medical School, Dallas, Texas).

Phage types 6, 52A, 42D, 44A, and 187 were used in testing grouping antisera A, B, F, G, and L, respectively. Neutralization serum constants (k) were determined at 37°C by the method described by Adams(8). Phages were diluted to approximately 2×10^5 PFU/ml and mixed with an equal amount of appropriately diluted antiserum. For a comparison of k values, antisera were diluted so that 65% (or greater) phage inactivation was obtained within 30 minutes.

The burst size and minimum latent period for phage UC-18 were determined from one step growth curves conducted at 37°C. A multiplicity of infection of 3 and 1-2 $\times 10^8$ cells/ml were used for all experiments.

Thermal inactivation was determined in broth (pH 7.2). The experiments were started by adding 0.5 ml of appropriately diluted phage to 49.5 ml of broth so that 50 ml of phage of approximately 5×10^5 PFU/ml were tested. A similar flask at room temperature served as a control. Both test reactants were equilibrated to the desired temperature before starting the experiment. Suitable aliquots were removed, cooled immediately in an ice bath, and plated. Inactivation rates were followed for 60 minutes.

Results and discussion. The morphology, general structure and size of phage UC-18 was similar to that of phages belonging to serological group B. Phage UC-18 has the head silhouette of a regular hexagon about 60 mμ in diameter and a comparatively short tail (about 165 mμ in length) terminating in a knob-like structure (Fig. 1B). The cross striations in the tail are evident. Bradley(9) has suggested that the terminal tail structure is a form of base plate based upon resolution of the knob showing appendages. Similar appendages on a tail knob, presumably taken on end, are shown in Fig. 1A.

Since most of the serum neutralization curves deviated from first-order kinetics it was necessary to use the initial rate of inactivation in determining the velocity constant (k) for each phage-antiserum mixture. Except for the group B antiserum, the k values for each antiserum were calculated from neutralization curves obtained using homologous phage. Phage 52A, a heterologous phage, was used to determine the k value of the group B antiserum because of difficulties encountered in visualizing plaque formation with phage 29. Although a homologous phage was not used in this latter case it was still possible to establish the relative neutralization rate of phage UC-18 as compared to other group B phages.

Phage UC-18 was neutralized by both group B and G antisera at comparative k ratios (k value of grouping antiserum/k value of grouping antiserum when tested with phage UC-18) of 40/20 and 210/100, respectively. Phages 52A and 44A, representing the phages of serologic groups B and G, were also neutralized by UC-18 antiserum.

Staphylococcal phages have been previously grouped by serologic methods(10,11). Other workers have found that phages of a

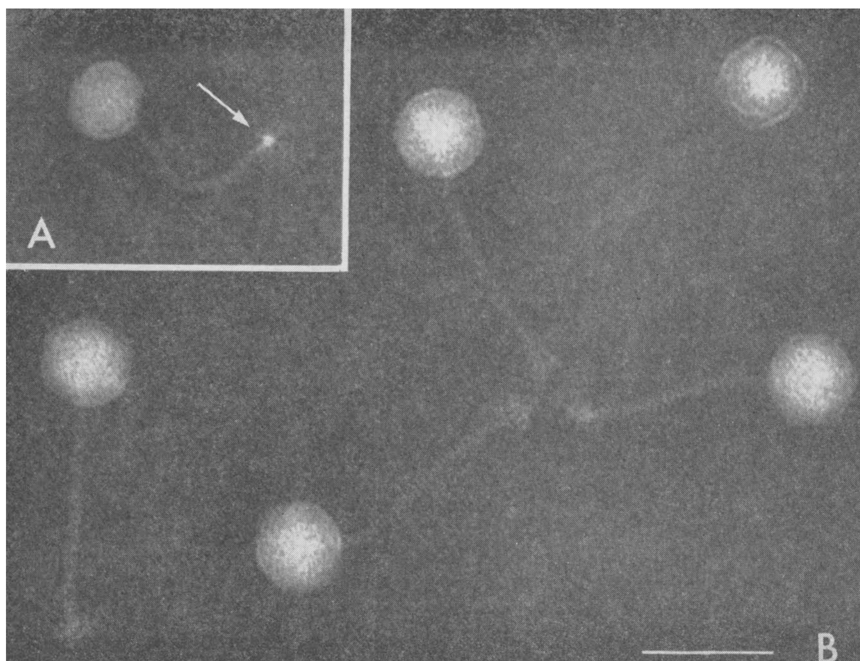

FIG. 1. A. Negatively stained preparation of phage UC-18 showing appendages at base of tail. B. Phage particles with regular hexagonal outlined heads. (× 180,000). Bar represents 100 mμ.

given serologic group have similar properties, and that these properties distinguish the phages of one group from another (9,10,11, 12,13). Recently, Rosenblum and Tyrone (14) extended these correlative properties to include buoyant density. In their studies the mean buoyant density for the phages comprising serologic group B was 1.502 g/cm^3. The mean buoyant density for phage UC-18 was 1.508 g/cm^3 and is consistent with their findings for this group. However, there exists an apparent antigenic relationship between phage UC-18 and the group B and G phages as noted previously. A similar observation concerning the antigenic relationship between some of the group B phages and 44A, a group G phage, has been reported by Rountree (10). The group G phages have been described as small phages unique in their apparent lack of tail structure with a buoyant density range of 1.457 to 1.459 g/cm^3 (14). In view of the marked differences that exist between phage UC-18 and the group G phages in morphology and buoyant density, it would seem appropriate to place UC-18 within the serologic group B phages.

The results of one step growth experiments with phage UC-18 indicated an average burst size of 7 PFU/cell and a minimum latent period of 35 minutes. During preliminary trials to establish the parameters of the burst size and latent period, it was found that the adsorption of phage UC-18 was quite slow as indicated by a relatively prolonged rise period. No attempt was made to determine the actual adsorption rate as the use of a higher multiplicity of infection circumvented this problem. The low yield and slow adsorption rate with phage UC-18 may reflect a nutritional or divalent cation requirement. This finding would not be surprising since the serologic group B typing phages have been shown to have small burst sizes and require divalent cations for adsorption or multiplication.

The results of thermal inactivation studies indicated that phage UC-18 was susceptible to temperatures of 45° to 55°C. Only 25% of the infective particles (based upon input) survived exposure for 60 minutes at 45°C, while at 55°C essentially all infective particles (>99%) were inactivated within 5 minutes.

Deviation from first order kinetics was observed when survivor rates were greater than 10% with inactivation curves obtained at 45°

to 50°C. This observation was further investigated to determine if the second component of the biphasic curve was due to a heterogenous phage population consisting of a relatively small proportion of thermally resistant particles(15). A single plaque obtained by plating phage exposed to 45°C and 50°C for 60 minutes was cored and the phage eluted in broth. Each stock was adjusted to 5 $\times$ 10^5 PFU/ml and again exposed to 45°C and 50°C. Essentially the same biphasic curves and rates resulted, thus indicating that the survival curves were due to a homogenous phage population.

Thermal inactivation for the basic series of staphylococcus typing phages has not been studied in sufficient detail to warrant a valid comparison of the inactivation results obtained with phage UC-18 and those of the other typing phages. However, Rountree(10) has reported a 5-10% survivor rate for the group B phages exposed to 49°C for 60 minutes. A 10% survivor rate was observed with phage UC-18 under similar test conditions and indicates a degree of similarity between phage UC-18 and those group B phages studied by Rountree(10).

Summary. Phage UC-18 was shown to have a latent period of 35 minutes and a burst size of 7 particles per cell. The phage had an average buoyant density of 1.508 g/cm^3 and on electron microscopic examination the head was found to have a regular hexagonal silhouette (60 mμ in diameter) with a tail (165 mμ in length) terminating in a knob-like baseplate with appendages. Antigenically phage UC-18 appears most closely related to the group B staphylococcal phages. Additionally, the phage was markedly sensitive to heat at 45° to 55°C. Comparison of the properties of phage UC-18 with those reported for the basic series of typing phages suggests that UC-18 should be included within the group B phages.

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Influenza B in the Spring of 1965. (31647)

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A controlled field study(1) was carried out by our group during the respiratory disease season of 1964-65 to evaluate the protective efficacy of a heptavalent respiratory agent vaccine which contained respiratory syncytial, parainfluenza 1, 2 and 3, and influenza A2 and B viral antigens and *Mycoplasma pneumoniae.* The investigation was carried out in 407