fected animals naturally leads to its possible application for diagnosis in patients, when test animals usually employed such as guinea pigs and white rats were not available. Preliminary studies in this connection have offered some indication as to its practical use. The fact that death is regularly produced in white mice by the yolk sac cultivated rickettsiae gives us an additional and convenient animal which may be employed for typhus studies. By its use, immunological and chemotherapeutic studies can be facilitated and extended.

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Urinary Excretion of Vi-Phage and *B. typhosus* Following Phage-Inoculation in a Typhoid Carrier.

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Following the finding of Vi-phage specific for V form of *B. typhosus,* the influence of phagotherapy on typhoid infections has gained new interest. Asheshov, Wilson and Topley[1] have demonstrated the protective effect of a Vi-phage on experimental typhoid infection in mice when the phage was given not later than 4 hours after infection. In the same experiment a non-specific phage, which could lyse both V and W forms *in vitro,* was found to be ineffective. Later, Fisk[2] also showed the protective action of a typhoid phage on experimental typhoid infection in mice. He, however, did not indicate whether the phage employed was a Vi-phage or not. In view of the lack of information on the influence of Vi-phage in human infection it is considered desirable, in this communication, to present some of our observations made on the treatment of a case of a urinary carrier of *B. typhosus* with Vi-phage and non-specific phages.

The patient (CCL), male, aged 42, with a history of symptoms of pyelitis, cystitis, and urethritis for at least 18 years, was first seen 3 years ago when *B. typhosus* was detected in his urine. Roentgenological examination then suggested the presence of bilateral renal calculi. In the succeeding 3 years, the patient was given vigorous local treatment with various urinary antiseptics such as argyrol, and oral medication with sulfanilamide and mandelic acid, but the

1 Asheshov, I. N., Wilson, J., and Topley, W. W. C., *Lancet,* 1937, **1**, 319.

2 Fisk, R. T., PROC. SOC. EXP. BIOL. AND MED., 1939, **38**, 659.

symptoms persisted and a type A V-form *B. typhosus,* as determined by the method of Craigie and Yen[3] was persistently isolated from the urine. During the same period the stool was repeatedly negative for *B. typhosus.*

The course of phagotherapy consisted of one subcutaneous injection of 1 cc of a combined phage followed by intramuscular injections of 2 cc of the same preparation at intervals of 2 to 3 days for 6 doses. The combined phage is a mixture of equal volumes of a potent type 2 Vi-phage of Craigie and Yen[3] specifically propagated on a type A V-form organism (P. 243) previously isolated from the patient's urine, and a non-specific "W"*-phage which lyses *in vitro* both V- and W-forms of *B. typhosus.* One cc of the combined phage contains 2.5×10^9 plaque units† of the Vi-phage and 3.5×10^8 plaque units of the "W"-phage. In addition to the phages, sodium bicarbonate was administered *per os* to maintain the alkalinity of the urine.

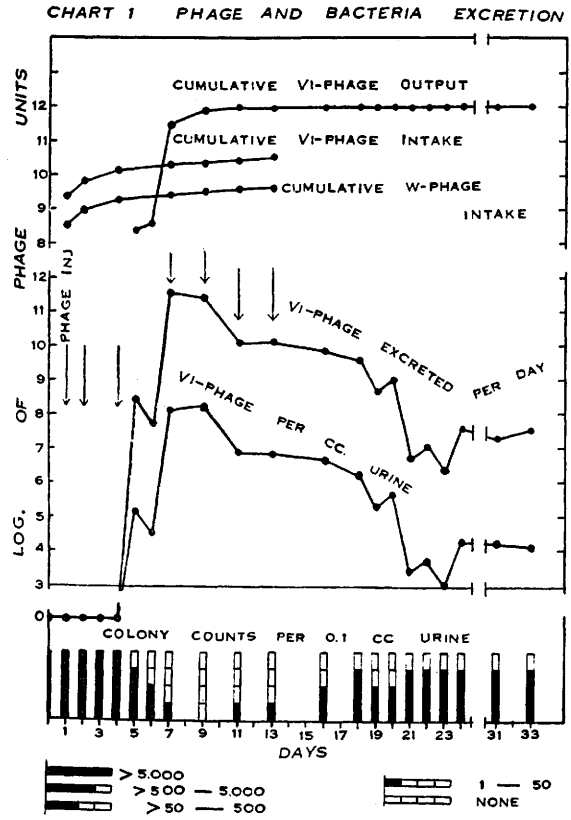
From 2 days before the first to 21 days following the last phage-injection, the urine and stool wehe repeatedly examined for the presence of antityphoid phages and *B. typhosus.* The stool consistently failed to show the presence either of antityphoid phage or *B. typhosus.* For studies of the urine, portions of non-catherized morning specimens were heated to 56°C for ½ hour immediately after collection and tested on plates previously flooded with susceptible organisms and freshly dried. For cultural studies the unheated portions of the same urines were inoculated on China-blue rosalic-acid medium. While it was impossible to ascertain that all the suspicious colonies obtained in these plates were *B. typhosus,* detailed examinations of a few of these from each specimen of urine examined have shown them to be *B. typhosus* as determined by fermentative and agglutinative tests. The results of daily excretion of Vi-phage and colony-counts of bacteria in the urine are summarized in Chart 1.

Results. The non-specific "W"-phage was not detected in any specimen of urine collected either before or after phagotherapy. This conspicuous failure to recover the "W"-phage from urine is interesting in the light of the lack of protective action *in vivo* of a non-specific phage reported by Asheshov, Wilson and Topley.[1] From the chart it can be seen that the Vi-phage began to appear in the urine in considerable quantity (1.4×10^5 plaques per cc) on the 7th-9th day;

[3] Craigie, J., and Yen, C. H., *Canad. Pub. Health J.,* 1938, **29**, 448.

* The "W"-phage was isolated by one of us (C. H. Y.) from a lysogenic typhoid culture in 1937 at Dr. Craigie's Laboratory, Toronto. This phage also causes lysis of *B. paratyphosus A, B, C; B. cholerœ suis,* and *B. aertrycke.*

† In this communication the units of phage are expressed in terms of the number of discrete plaques seen on agar-surface growths.



it then decreased significantly despite the fact that 2 more doses of phage were given and finally was maintained at a lower level (1.1×10^3-2.2×10^4 plaques per cc) for as long as 21 days after the last injection, at which time this observation was terminated. From the cumulative output and intake of phage and its daily excretion, it is evident that the Vi-phage must have multiplied within the body. Accompanying the first appearance of the Vi-phage in the urine there was a decided decrease in the colony-counts, and at the time when the output of Vi-phage was maximal there was actually a failure to obtain growth from the urine. Subsequently, as the concentration of Vi-phage in the urine became reduced, the colony-count again tended to increase, and from the 16th day onwards *B. typhosus,* while distinctly less frequent than before the treatment with phage, was persistently present. Coincident with the simultaneous presence of Vi-phage and V-form organisms in the urine, there appeared Vi-phage-resistant W forms and tiny colonies of a lysogenic form in the same specimens. These latter forms were not

seen in the urine before the appearance of Vi-phage. The Vi-phage-susceptible V-forms isolated either before or after the appearance of the phage in the urine consistently belonged to the bacteriophagic type A of Craigie and Yen,[3] but were frequently non-motile. The motility, however, could be regained after a few subculturings in broth. The V-form organisms isolated from phage-containing urine were just as susceptible to the action of the same specimen of urine as to the action of the original Vi-phage. The Vi-phage isolated from the urine was specifically neutralized by the anti-Vi-phage type 2 rabbit serum and behaved like a ϕ A-preparation of Craigie and Yen in its differential action on the standard type-strains. Thus the phage recovered from the urine is identical with the Vi-phage administered.

The simultaneous existence in the virus of Vi-phage-susceptible V-form organisms together with the Vi-phage is difficult to explain. When a fresh specimen of phage-containing urine (1.1×10^3 plaques per cc) was incubated at 37°C overnight the Vi-phage-concentration was found to increase by at least 10^5 fold and the resultant cultures isolated from this were Vi-phage-resistant W-forms which were still susceptible to "W"-phage. When the incubation was made anaërobically *in vacuo* the multiplication of phage was only 10 to 10^2 fold and the Vi-phage-susceptible V-forms could still be recovered from the urine. This suggests that the absence of free air in the urinary bladder may be a factor favoring the incomplete action of Vi-phage on V-form organisms. The intervals between two successive voidings were next investigated in order to see whether a longer interval would allow a more complete action of the phage on the organism within body. Table I gives one such determination. It will be seen that both phage- and colony-counts varied greatly on different specimens of urine passed within a period of 24 hours. There appears to be no simple correlation between the duration of time the urine was retained in the bladder and the amount of phage and bacteria excreted. But the highest phage-count associated with a

TABLE I.
Variation of Phage and Bacteria Counts in Different Specimens of Urine Collected within 24 Hours.

Date	Time	Time interval between 2 voidings	pH	Phage units per cc	Bacterial colony per 0.1 cc
April 21, 1940	7:40 A.M.	5 hr 5 min	8.4	1.3×10^8	5.0×10
"	9:45 "	2 5	8.4	8.3×10^7	1.0×10^2
"	1:50 P.M.	4 5	8.2	4.3×10^7	3.9×10^2
"	3:30 "	1 40	8.6	9.7×10^3	1.3×10^3
"	8:40 "	5 10	7.8	1.3×10^7	5.4×10^2
22	1:00 A.M.	4 20	7.0	2.6×10^4	9.0×10^2
"	7:05 "	6 5	7.6	2.2×10^4	8.7×10^2

low colony-count was seen when the urine was passed after an interval of over 5 hours, whereas the lowest phage-count associated with a high colony-count was seen in the urine passed after an interval of only 1½ hours. This lack of straightforward correlation may be due to the presence of other factors such as the variation in the number of bacteria discharged, probably intermittently, into the urine from the local lesion. However, neither the pH value, which varied from 6.8 to 8.6; the specific gravity, which varied from 1.015 to 1.021; nor the daily output of urine, which varied from 1.5 to 2.2 liters, showed any correlation with the amount of phage on the number of bacteria voided.

The blood of the patient examined on the 16th day of phagotherapy, revealed the presence of potent neutralizing antibodies against both the type 2 Vi-phage and the "W"-phage. The agglutinins of the same serum were as follows: O-1:320; H-1:640; Vi-1:40. Control serum from a typhoid convalescent (possessing agglutinins O-1:640; H-1:1280, and Vi-1:60) was found to contain no phage-neutralizing power. Thus the development of antiphage in the patient's blood appears to be unrelated to the presence of agglutinins.

The existence of phage-antibodies and the other data presented above seem to offer a plausible explanation for the simultaneous existence of Vi-phage and V-form organisms in excreted urine, if one postulates the existence of a focus of infection in the urinary system as seems more than likely to have been present in this case. In the focal lesion, the V-form organisms may have, being protected from the action of phage by the serum, and phage-susceptible organisms discharged intermittently into the bladder-urine where the Vi-phage could bring about lysis. The completeness of lysis would then depend upon the duration of contact, and relative concentration of phage and bacteria. The lytic action probably does not progress with maximal rapidity on account of lack of free air. The excreted urine would contain, therefore, not only the Vi-phage-resistant W-forms and lysogenic V-forms resulting from the action of Vi-phage on V-forms, but also the susceptible V-forms which are intermittently discharged from the lesion and have not been acted upon by the Vi-phage. The presence in the bladder of a small amount of urine after each voiding would serve to supply the Vi-phage after phage-injections were stopped. In this way a state may be produced similar to that observed, namely, the simultaneous presence of Vi-phage and Vi-phage-susceptible organisms in the same specimen of urine over a long period of time.

Summary. Following subcutaneous and intramuscular infections

of a Vi-phage and a non-specific antityphoid phage in a urinary typhoid carrier, only the Vi-phage appeared in the urine in amounts greater than those administered. The numbers of *B. typhosus* in the urine were transiently decreased as the result of phage-treatment but increased again considerably with the appearance of W-form and lysogenic V-forms in addition to the typical V-forms which had been persistently present. The presence of W- and lysogenic V-forms was attributed to the action of V-phage in the bladder-urine and the presence of the V-form organisms due to the intermittent discharge of organisms from a focus in which the action of the Vi-phage was probably inhibited. At the same time specific antibodies against both phages were developed in the blood. The type-specificity of the phage and bacteriophagic typing of the Vi-form organisms repeatedly isolated from the urine remained constant.

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Studies on Stability of Dilute Purified Tuberculins.

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Concentrated tuberculin is remarkably stable whereas diluted tuberculin usually undergoes rapid deterioration. By the use of alkaline buffer solutions as diluting agents, however, old tuberculin has been claimed to maintain its potency for 4 to 6 months.[1, 2, 3] On the other hand, when the same diluents were applied to purified tuberculins by the same authors less favorable results were obtained; deterioration of the product generally occurring in 4 to 6 weeks. It is evident that a suitable diluent has not been found. In the present report a phosphate buffer of pH 7.0 is described which in our hands has been found to maintain the potency of dilute purified tuberculins for at least 4 to 6 months.

Materials and method. The preparation of the purified tuberculin employed for the study was similar in principle to that of Seibert's PPD. Its potency and other properties will be reported elsewhere. For the present it is sufficient to state that comparative tests made on

1 Douglas, S. R., and Hartley, P., *Tubercle*, 1934, **16**, 105.

2 Gottschall, R., and Bunney, W. E., *J. Immunol.*, 1938, **34**, 103.

3 Jensen, K. A., Bindslev, G., Moller, S., Hansen, A., and Lind, P., *Tubercle*, 1938, **19**, 385.