polysaccharides A or with the polysaccharides reported previously.[1] Likewise, cross absorption tests performed by mixing the same serum with organisms belonging to any of the heterologous types did not effect the precipitin titer nor the type-specific agglutinins.

The above findings indicate that 2 distinct kinds of polysaccharide are present in the so-called different serological types included in this study. One type of polysaccharide (A) appears to be shared by at least 4 of the 5 types while the second (B) has been encountered only once. The question of whether or not more than 2 types of polysaccharide are present in other serological types of *C. diphtheriæ* is being investigated.

10605

Bacteriophage Typing of *B. typhosus* Isolated in Peiping.

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Four serologically distinct types of Vi-phages (Type I, II, III, and IV) specific for V-form strains of *B. typhosus* have been described.[1, 2] Type II Vi-phage was found to exhibit a highly selective lytic activity for the strain of *B. typhosus* from which it was propagated. Utilizing this selective behavior of the Type II Vi-phage a method has been evolved whereby V-form strains of *B. typhosus* could be divided into the typical V-forms and the Imperfect V-forms. The typical V-forms were further subdivided into several distinct types (A, B1, B2, C, D1, D2, E, F1, F2, G, H, J).[2] Studies on 706 strains of *B. typhosus* isolated in Canada, England, Norway, Sweden and Denmark have demonstrated the validity of such a scheme of typing.[3] In order to test further the applicability of this scheme of bacteriophage typing and to determine the incidence of different types of *B. typhosus* occurring in this locality, a study was made on 79 strains isolated in Peiping. The results together with an account of isolation of 2 new type strains are given below.

Seventy-nine strains of *B. typhosus* isolated from active cases and carriers during 1937-1939 were inoculated in semisolid agar media

1 Craigie, J., and Yen, C. H., *Trans. Roy. Soc. Canada*, 1937, **31**, Sect. V, 79.
2 Craigie, J., and Yen, C. H., *Canad. Pub. Health J.*, 1938, **29**, 448.
3 Craigie, J., and Yen, C. H., *Canad. Pub. Health J.*, 1938, **29**, 484.

and kept in the refrigerator (0°-6°C). Subculturing was carried out every 3 months. In the present study these stock cultures were plated out on nutrient agar plates (1.5% agar, pH 7.6) and single colonies were picked and employed for typing. These cultures thus derived were then stocked in the nutrient agar stubs, instead of semisolid agar. The original method[2] of typing with Type II Vi-phage preparations* was strictly followed.

Out of a total of 79 strains studied 74 strains of V forms were isolated. Of these 74 strains 23 belonged to the "Imperfect V-forms" and 51 to the typical V-forms. The distribution of the types in these 51 strains are given in Table I. It is to be noted that among the typical V-forms isolated in Peiping only A, D1, and E types were encountered. In addition 2 new types (P16 and P15) were isolated.

Both P15 and P16 strains were found to be insusceptible to the critical phage dilution test[2] of known standard Type II Vi-phage preparations. The Type II Vi-phage was propagated on P16 and P15 separately for numerous generations until the initial phage had been actually diluted beyond 10^{31} folds. These phage preparations were designated as α16 and α15 respectively in accordance with the nomenclature used before.[2]

The critical dilution[2] of 16 was unable to cause a complete area of lysis on plates in any of the known type cultures except Types A and P16. Therefore P16 represents a new type. Subsequently it was found that 6 other local strains belonged to the P16 type.

TABLE I.
Distribution of the Types of *B. typhosus*.

	Typical V types					Imperfect		
	A	D1	E	P15	P16	V-forms	W-forms*	Total
No. of strains (a)	11	5	24	4	7	23	5	79
No. of individuals (b)	8	4	12	3	6	11	5	49
Times isolated per individual (c)								
1	5	3	6	2	5	7	5	33
2	3	1	3	1	1	2		11
3			2			1		3
6			1					1
9						1		1

(a) No. of strains belonging to each type.
(b) No. of individuals from whom the above strains were derived.
(c) No. of individuals from whom the isolation has been made.
*Strains of *B. typhosus* devoid of Vi antigen and insusceptible to Vi-phages.

*We are indebted to Dr. James Craigie of the Connaught Laboratories, Toronto, Canada, for supplying us the original stock of the standard "V" Type cultures and the Type II V-phage preparations employed in this study.

TABLE II.
Lytic Activity of α15.

Culture types	Phage Dilutions			
	10^{-2}	3×10^{-2}*	10^{-3}	10^{-4}
P15	C	C	++++	+++
A	C	C	C	C
B1	C	C	C	+++
B2	C	+++	++	+
C	C	C	C	+++
P16	C	C	++	+

C = Whole area lysed.
++++ = Isolated plaque numerous.
+++ = 25-50 plaques.
++ = 20-25 plaques.
+ = less than 10 plaques.
* = critical dilution of α15.

When the critical dilution of α15 was tested on the known standard type strains, it was able to cause a complete area of lysis on the plates not only for Types A and P15 culture but also for B1, C, P16 and to a lesser degree B2, E, G, and H cultures. In order to determine the relationship of α15 to other standard Type II Vi-phage preparations, serial dilutions of this phage were tested on the strains of standard type that are comparatively susceptible to this phage. The results are given in Table II.

It is obvious that α15 seem to be more potent for the cultures of Types A, B1, and C than for P15. This is due to the fact lysis of P15 by α15 results in tiny plaque formation and the lysis is delayed. In fact the lysis becomes more pronounced when the plate cultures of P15 instilled with α15 was first incubated at 37°C overnight followed by leaving at room temperature for another 24 hours. On account of delayed lysis and small size plaque formation the critical dilution of α15 is only as dilute 3×10^{-2}. Thus the properties of P15 and α15 do not resemble any of the known types reported.[2] It was, therefore thought convenient to designate P15 as a distinct type for differentiation from others. Subsequent typing of the local strains revealed that 3 other strains belonged to this new type.

Strains of *B. typhosus* isolated from the same individuals at different times always fall into the same type. Thus a patient (H. 35952) consistently gave isolation of E type strains 6 times and another patient (H. 64490) gave isolation of "Imperfect V form" strains 9 times. Similarly V forms of *B. typhosus* obtained from various sources (stool, blood, urine, bile, rectal swab and chest-wound swab) from the same individual always belong to the same type.

These results clearly indicate the practicability of the method for typing *B. typhosus* by bacteriophage.[2] It is of interest to note that relatively fewer types of typical V form strains were encountered in this locality in comparison with those reported in other countries. Studies are being continued to define the two new type strains (P16 and P15) described.

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Type Stability to Bacteriophage of Variants of *B. typhosus.*

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During a study made on the typing of local strains of *B. typhosus,* colonies of various appearances were noted on plating of these old cultures. This afforded the opportunity of determining by the bacteriophage-typing technic[1] whether or not various kinds of colonies derived from a parent stock culture would belong to the same type of V-forms. The observations made regarding this point are herewith communicated.

A collection of 79 strains of *B. typhosus* reported previously[2] was studied. These cultures were kept in semisolid agar media for 3 months to 1½ years with subculturing every 3 months. Upon spreading of these stock cultures on nutrient agar plate (1.5% agar, pH 7.6) followed by incubation at 37°C for 16-20 hours the following kinds of colonies were encountered:

A. Normal Colonies.
   1. Round Margin, Opaque.
B. Variant Colonies.
   2. Round Margin, Translucent.
   3. " " Mosaic.
   4. Rough " Opaque.
   5. " " Translucent.
   6. " " Mosaic.

All normal and variant colonies have smooth surfaces and their suspensions in 0.9% saline are stable. They, however, vary greatly in regard to the colony outline, opacity and size. Thus the colony outline may be smooth and round or irregular, rough with fan-like

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

[2] Yen, C. H., PROC. SOC. EXP. BIOL. AND MED., 1939, in press.