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.

10606

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

C. H. YEN. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

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.

margin, the opacity may be opaque, mosaic or with varying degrees of translucency and the colony size may vary from 1 to 5 mm in diameter. On repeated subculturing the variant colonies showing irregular margins may become smooth and round, and the translucency or mosaic appearance may revert to that of normal opacity. Thus no complete or stable rough colonies have been encountered. These colonies may be regarded as colony variants probably degradation of S form with varying degrees of roughness.

Of 79 strains studied, 27 strains gave only the normal colonies, 3 strains only the variant colonies and the remaining 49 strains gave mixtures of normal colonies with one or more kinds of variant colonies. The result of typing of the normal colony strains has been analyzed in a previous communication.[2] The 3 strains that gave only the variant colonies were also included in that report. These 3 strains were typed on the basis of the sensitivity to the Type II Vi-phage preparations and were found to belong to an Imperfect V form, a Type A type and a P15 type separately.

The results of typing of the normal and variant colonies occurring together from 49 strains of stock cultures are summarized in Table I.

From Table I it is to be noted that when the normal colonies fall into the W, VW, or Imperfect V forms, the variant colonies derived from the same culture also fall into the same forms. But when the normal colonies are of typical V forms, the variant colonies may belong to W, VW, or typical V forms. All the V forms derived from the variant colonies, however, when tested with Type II Vi-phage preparations, were found to belong to the same type as that of the normal colonies, arising from the same culture. There was not a single instance in which the normal colony is of one typical V type and

TABLE I.
Distribution of Types Found in 49 Strains Giving Both Normal and Variant Colonies.

No. of strains	Normal colony	Variant colony
3	W form	W form
17	Imperfect V form	Imperfect V form
2	VW form (Type A and W)	VW form (Type A and W)
3	Type A	W form
5	" A	Type A
1	" D1	VW form (Type D1 and W)
1	" D1	Type D1
3	" E	W form
8	" E	Type E
1	" P15	VW form (Type 15 and W)
2	" P15	Type P15
1	" 16	" W form
2	" P16	" P16

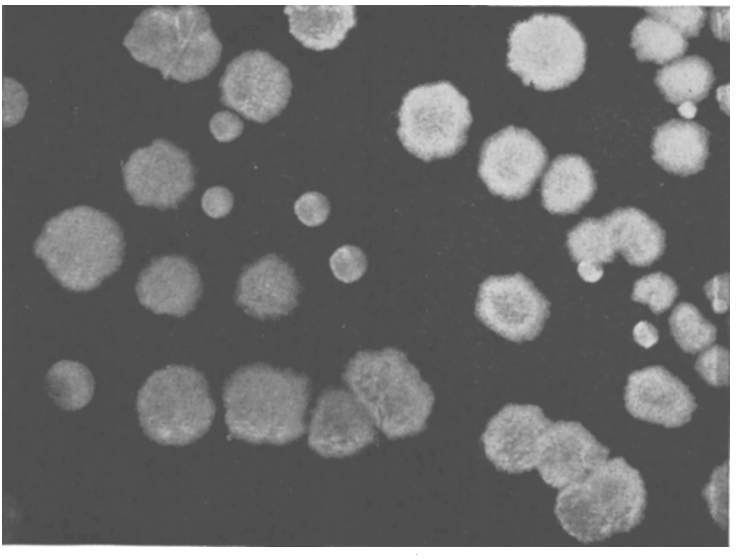

FIG. 1.

Strain P4. 24 hours growth of *B. typhosus* on nutrient agar at 37°C. Variant colonies larger than normal one. Note irregular margin and mosaic appearance of the variant colonies. × 4.

the variant colony of another typical V type. Nor was there any instance in which the variant colony falling to one typical V type while the normal colony from the same culture falling to W or VW forms. Illustrations are given in Fig. 1 showing a mixture of normal and variant colonies belonging to Type A derived from the stock culture P4.

After reversion of variant colonies to normal appearances on subculturing, the type identity of the cultures remained unchanged. It may be pointed out here that the colony appearance is not a reliable guide to indicate whether a colony is a V form or a W form. But W forms are more frequently encountered in the variant colonies than in the normal colonies.

Thus it is clear from these observations that the change of colony appearance from normal smoothness to various degrees of roughness may or may not be accompanied by a loss of the Vi-phage susceptibility. As long as the Vi-phage susceptibility is present in the variant colonies they can be typed with the bacteriophage technic proposed by Craigie and Yen.[1] The fixity of the types defined by the method is well borne out by the fact that the variant colonies when still susceptible to the Vi-phage always fall into the same type as the normal colonies derived from the same culture.