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Dissociation of *V. Cholerae* by Bacteriophage.

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Bacteriophage is known to exercise a strong influence in enforcing microbic dissociation of certain bacterial cultures. Bacteriophage on *V. cholerae* produces phage resistant secondary cultures *in vitro* and certain coccoid mutants in convalescent cases (d'Herelle¹). The variation of *V. cholerae* has also been reported by Baerthlein² Balteanu,³ and others. The object of this study is to determine whether dissociation of *V. cholerae* by bacteriophage follows the same cycle proved for other motile bacteria, such as *B. paratyphosus A* by Arkwright⁴ and hog-cholera bacillus by Li.⁵ Morison's⁶ combined phage of *V. cholerae* and *B. dysenteriae Shiga* was used for inducing dissociation. Two strains of *V. cholerae* susceptible to the phage were selected for study. One cc. of an 18-hour peptone water (pH 8.0) culture of each was transferred to a fresh peptone water medium to which half cc. of Morison's phage was introduced and incubated at 37°C. for one week. The process was repeated at weekly intervals, the cultures diluted, plated on semi-solid medium for examination of motility, and streaked on agar plates for the character of the surface colonies.

Variation in the appearance of the colonies appeared in the semi-solid plates after 2 to 3 transfers followed gradually with more advanced changes as the transferring process went on, while the control tubes invariably gave one type of MS (motile smooth) colonies which grew deep, homogenous, similar to the smooth surface colonies on the agar plate (Fig. 1). Three distinct main types of variants arose from the phage resistant secondary cultures. After 2 to 4 weeks the first variant appeared from the secondary cultures as an irregularly shaped colony with clusters of small daughter colonies in the semi-solid medium (Fig. 2). The individual cells

¹ d'Herelle, F., Malone, R. H., and Lahiri, M. N., *Ind. J. Med. Res. Memoirs*, 1930, **14**.

² Baerthlein, K., *Cent. f. Bakteriolog. I. O.*, 1918, **81**, 369.

³ Balteanu, J., *J. Path. and Bact.*, 1926, **29**, 251.

⁴ Arkwright, J. A., *J. Path. and Bact.*, 1927, **30**, 345.

⁵ Li, C. P., *J. Exp. Med.*, 1929, **50**, 245.

⁶ Morison, J., and Vardon, A. C., *Ind. J. Med. Res.*, 1929, **17**, 48.

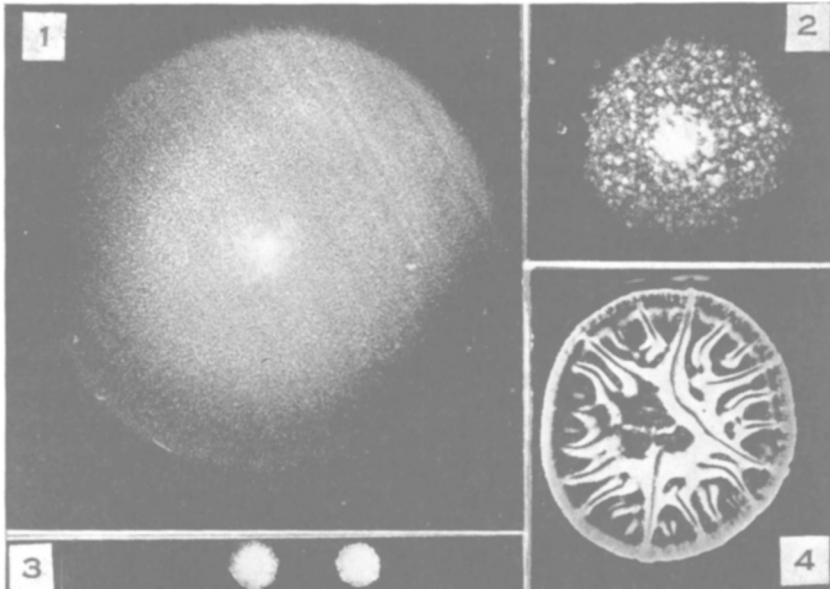


FIG. 1. Normal MS colony of *V. cholerae* 18 hours old, growing in semi-solid medium. $\times 15$.

FIG. 2. MR colony 18 hours old in semi-solid medium, showing the cluster formation. $\times 15$.

FIG. 3. NS colonies 48 hours old in semi-solid medium. NS and NR colonies appear alike under these conditions. $\times 15$.

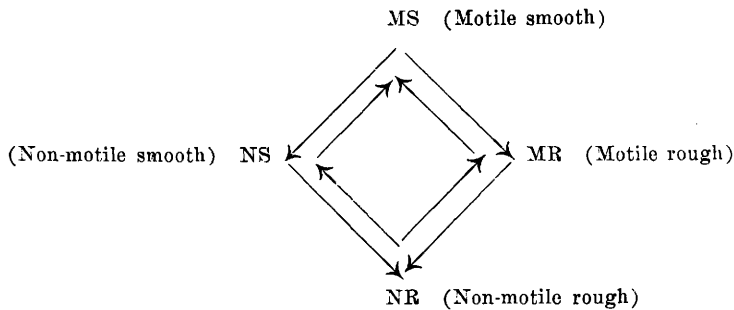
FIG. 4. NR colony 24 hours old on agar plate. MR and NR surface colonies appear alike under these conditions. $\times 15$.

were motile but they formed typical rough, opaque surface colonies on agar plate and constituted the MR (motile rough) colonies. After 4 to 6 weeks the second variant appeared as very small compact colonies in the semi-solid medium, but formed typical surface smooth colonies on agar plate. They were non-motile and termed the NS (non-motile smooth) colonies (Fig. 3). After 6 to 10 weeks the third variant appeared as deep colonies similar to the NS type in semi-solid medium, but gave rough surface colonies similar to the MR type on agar plate (Fig. 4). It was the most stable variant and named as NR (non-motile rough) colony. Both rough variants gave granular growth in broth and were spontaneously agglutinated in saline, while both smooth forms grew homogeneously in broth and formed stable suspensions in saline.

By daily transfers on agar slants the MR type showed a tendency to revert to MS type after 46 transfers; the other 2 types remained stable. But when the variants were repeatedly grown in peptone water and transfers were made twice a day for 30 days all the variants showed a tendency to reversion. Typical MS colonies

appeared in the MR culture after 34 transfers in peptone water (twice a day) and also a few motile ones from the non-motile types after 60 transfers (NS to MS and NR to MR). Reversion of the variants was more evident by animal passage than by laboratory media. The MR culture showed some reversion to smooth after 6 animal passages, while after 10 passages some MS colonies appeared in the NS culture and both MR and NS forms from the NR culture.

This study seems to suggest the NR type is the direction towards which dissociation of *V. cholerae* leads and that the MR and NS forms are simply intermediate types. All the MS forms induced back from the variants by any process are as susceptible to the same phage action as the original culture. The dissociation induced by bacteriophage and the reversion of variant by animal passage as observed may be represented by the following diagram:



Organisms from all the 4 types were gram negative slender rods and culturally were typical. Virulence tests on hamsters (Chinese field mice) showed that, the variants were comparatively less virulent than the original MS culture, especially the NR type which was practically avirulent. Serologically there was very slight difference among the 4 forms except that the NR type gave an agglutination of a very low titer with the anti-sera of the 4 types.