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Studies on Variability of *B. diphtheriae*.

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Typical cultures of virulent diphtheria bacilli grown in broth containing 10% specific antibacterial serum gave origin to distinct and rather stable variant types differing markedly from the parent strain in colony form, lacking in fermentative properties and in virulence and toxigenicity. Likewise, continued cultivation of typical cultures (at 37°C.) in broth containing 0.1% phenol, of 0.5% lithium chloride or 2.5% pancreatin as well as cultivation in K-medium (Kendall) diluted 1:3 with physiologic salt solution and cultivation in plain broth at 45°C., gave rise to the development of similar variant types.

In some instances transformation from the original characteristic colony type to the variant type occurred suddenly while in other instances certain variations or intermediate changes in colony form with loss of virulence were noted before the appearance of the extreme variant type. The intermediate types probably corresponded to those noted by other observers of bacterial dissociation in *B. diphtheriae* cultures.

The variant or 'V' colonies are smaller, less opaque and grow less rapidly on various culture media. In morphology the 'V' organisms are pleomorphic, exhibiting small coccoid or occasionally somewhat elongated solid staining types. Broth cultures develop a uniform turbidity which is slow in appearing. The 'V' cultures are practically devoid of fermentative ability. Guinea pigs are not affected by inoculation with living 'V' cultures or with broth filtrates, indicating loss in virulence and in toxigenicity incident to the forced transformation.

These properties, which characterize the 'V' type culture have remained constant in cultures maintained over periods of 6 months to 2 years indicating considerable stability of the variant type.

Antisera containing agglutinins for the mother-type organisms were devoid of agglutinating effect upon some of the 'V' strains while other 'V' strains were agglutinated, indicating that agglutigen common to the parent organism might be variably transmitted to the variant. In many instances variants apparently were dissimilar antigenically since antisera prepared against a particular

variant frequently failed to agglutinate to any considerable extent other variants derived from the same parent organism.

Since there was no evidence that the variant type would spontaneously revert to the parent type organism an attempt was made to determine whether or not reversion from variant to original colony type, etc., could be induced by certain types of *in vitro* and *in vivo* cultivation, as has been accomplished with the rough variants of pneumococci, paratyphoid bacilli and other organisms. Accordingly the following technics were employed: *a.* variant cultures were transplanted in nutrient broth at 24-hour intervals over periods of 20 days, *b.* cultures were made in broth containing heat-killed organisms of the respective original type, *c.* cultures were made in broth containing heat-killed organisms of the parent type plus 5% of the 'V' antiserum, *d.* living 'V' type organisms were injected into guinea pigs, intraperitoneally, with heat-killed organisms of the original type. Cultural and pathogenic properties were not changed incident to these modes of cultivation and reversion in colonial form occurred only in one instance, wherein, one of the variants was injected into a guinea pig with heat-killed organisms of the original type and plates inoculated after 24 hours with the peritoneal fluid showed an admixture of parent-type with variant-type colonies. The former were lacking in pathogenicity and were quite unstable, readily dissociating into the 'V' type upon subculturing.

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Production of an Unsaturated Compound by *B. coli* in a Synthetic Medium.

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Production of unsaturated acids by bacteria in synthetic media has apparently rarely been observed. When *B. coli* were grown in a media* of known composition, it was noted that acidity continued

* Synthetic Media 22.	gm.		gm.
Dextrin	10.0	Calcium Chloride	0.1
Potassium Hydrogen Phosphate.....	1.0	Ammonium Tartrate	10.0
Potassium Di-hydrogen Phosphate..	1.0	Distilled Water	1000 cc.
Magnesium Sulphate	0.1		

pH, 8.6. Sterilized at 10 lb. for 15 minutes.