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.

6255

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

W. F. LANGE. (Introduced by M. S. Fleisher.)

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

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.

to increase even when tests showed the absence of lactic, acetic and aceto-acetic acids, and it was noted that the media readily absorbed bromine and iodine and decolorized potassium permanganate.

Cultures were made in large quantities of the media and allowed to grow for a period of 20 days. If tests for dextrin, reducing sugars, lactic acid ammonia, nitrites and nitrates were negative, the test for tartrate ion (alpha-beta-hydroxy-acid)† was less marked than in the control media and the pH was 5.0 or lower, the media was freed of the organisms by filtration through a Seitz filter. The filtrate was extracted 2 or 3 times with ether (free from peroxides). The ether was distilled from the extract. The residue, a water-clear liquid, was dissolved in a small quantity of water and reextracted with ether. The ether from the second extraction was removed by spontaneous evaporation in the dark, since light seemed to cause polymerization of the residue.

The residue, a water-like, colorless liquid, as stated above had the following qualities:

A strong acetic odor; readily soluble in water, ether, alcohol, and chloroform, a melting point of about 6°C. It was readily decomposed by sunlight but more slowly by diffuse light. Some of the extract was placed in a small vial, was kept in the icebox, but even under these conditions the material decomposed within 3 days.

It was found that 0.08 cc. of the liquid when dissolved in 3 cc. of 10% sodium carbonate solution decolorized more than 8 cc. of a 1% solution of potassium permanganate leaving a brown precipitate of manganese oxides; 0.13 gm. of the liquid dissolved in 2 cc. of carbon tetrachloride to which was added 3 drops of brom-solution (2 cc. of bromine to 50 cc. carbon tetrachloride) caused the decomposition of the bromine solution instantaneously without the evolution of hydrobromic acid. The decolorization of the brom-solution continued until 8 cc. had been added. When the solution was warmed an additional 2 cc. of the brom-solution was decolorized.

The decolorization of potassium permanganate and the absorption of bromine show that the residue is an unsaturated compound which may contain one or 2 unsaturated bonds. The large amounts absorbed seem to indicate that the compound has two unsaturated bonds.

---

† The test here used was the formation of the copper complex ion, which is not specific for tartrate ion but may be obtained by any alpha-beta-hydroxy-acid. The product isolated from the media responds to this test and in all probability was responsible for the positive results.

The reaction with heavy metals is usually regarded as specific for propiolic acid when the odor, solubility, melting point, instability, and unsaturation have been established. A greenish yellow precipitate was formed at once when 1 cc. of a standard ammonical cuprous chloride solution was diluted with 5 cc. of distilled water and added to a drop of the ether extract. A whitish-yellow precipitate was formed which turned dark within a few seconds and left a mirror-like deposit in the test tube when silver nitrate was added to the aqueous solution.

The number of hydrogen atoms replaced by silver in this reaction is problematic. The instability of this compound makes it impossible to obtain accurate results. However, the summation of numerous determinations showed approximate results which indicated that one hydrogen atom of the ether extract was replaced by one silver atom after which decomposition occurred, leaving the mirror-like deposit. This unsaturated compound does not appear in the media in large quantities. It requires 12 liters of the media to obtain 1.06 gm. or 0.088 gm. per liter.

The product extracted from the media is very much like propiolic acid.[1] The purity of the material tested may be questioned in view of the limited attempts made to purify the substance. It is, however, very unstable and could be obtained only in such small quantities as to make further chemical analysis very difficult. Yet all tests applied to the substance clearly and distinctly indicate a pure product. The assumption that this acid is propiolic is based on the results obtained when tested as described by Mulliken.

It cannot be definitely stated that this acid is produced in the metabolism of the carbohydrates since during the lengthy period of growth some of the bacteria may have disintegrated and might thus be responsible for this product. If this were true the amount of acid should increase with time; however the best yields were obtained soon after tests for dextrin and the tartrate ion became negative and thereafter the substance gradually disappeared from media (25-30 days after inoculation).

When ammonium chloride is substituted for ammonium tartrate in the media, an alpha-beta-hydroxy-acid appears in the media between the 8-13 days and then disappears. This acid when extracted with ether responds to the same tests as that isolated when tartrate is used instead of chloride.

While it cannot be definitely stated yet it seems rather conclusive

---

[1] Mulliken, Identification of Pure Organic Compounds, 1920, 75. Beilstein, *Handbuch der Organ. Chem.*, 1920, **2**, 477.

that this unsaturated acid is an intermediate product in the metabolism of dextrin.

The product isolated has been shown to be an unsaturated acid which in every way corresponds to propiolic acid.

6256

Reversibility of Specific Adsorption of Bacterial Agglutinin.

MALCOLM H. MERRILL. (Introduced by Moyer S. Fleisher.)

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

The adsorption of agglutinin by specific organisms in the presence of low concentrations of electrolytes is a reversible reaction. This has been shown by the following experiment: Immune serum was diluted 1 to 50 with distilled water and added to distilled water suspensions of *B. coli* throughout a pH range of 2.5 to 10.5. Maximum adsorption as determined by titration of the supernatant after centrifuging occurs at pH 5.8 to 6.0, the adsorption becoming progressively less with either increasing acidity or alkalinity. At pH 3.0 and 10.5 adsorption was always less than 5% compared to 98 to 99% at pH 6.0. Washed organisms were used for adsorption and for the agglutinin determination.

When adsorption occurred at pH 6.0 and the sedimented bacteria were resuspended in distilled water at various pH values a reversal of adsorption occurred in both acid and alkali. The titer of these extracts was always distinctly lower than was the residual titer of the supernatant following adsorption at the same pH. If, however, the eluted extracts were mixed with the supernatant obtained after adsorption at pH 6.0, from which over 98% of the agglutinin activity had been adsorbed, the titer was now restored to that of the supernatant after simple adsorption at the corresponding pH. The addition of normal rabbit serum to the extracts to give the same content of normal serum constituents exerted the same effect.

The work demonstrates that reversal of adsorption is possible and indicates the dependence of agglutination upon the presence of normal serum constituents. This last factor appears to have been neglected by previous workers who have studied the reversibility of agglutinin adsorption.