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Influence of an artificial peroxidase upon the growth of anaerobic bacilli.

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Previous study of the influence of plant tissue upon bacterial growth showed that sterile unheated plant tissue can replace blood in the cultivation of the so called hemophilic organism *B. influenzæ*. It was also found that plant tissue exerts a marked accelerating action upon the growth of pneumococcus. More recently it has been shown that the addition of unheated vegetable (potato) to plain bouillon makes possible the continued, ærobic growth of certain anærobic bacilli such as *B. histolyticus, B. ærofetidis, B. oedematiens,* and *B. chauvei.*

This growth promoting action upon three distinct groups of bacteria so widely different in their cultural requirements, indicates that plant tissue meets certain physiological needs of the bacterial cell not wholly provided for by the ordinary culture media. These studies suggest that this action may be dependent not only on the presence of some growth accessory substances in plant tissue but also upon the functioning of certain systems which are concerned in the cellular processes of oxidation and reduction.

McLeod and his associates have found that certain ærobic bacteria which are devoid of catalase form hydrogen peroxide when grown in the presence of air. In the case of pneumococcus, which possesses no catalase, hydrogen peroxide is known to accumulate in the fluid of ærobic cultures in concentrations which are bacteriostatic and even bacteriocidal. As far as is known anærobic bacilli are also devoid of catalase, and hence these cells cannot destroy peroxides. From these relations it seems not unlikely that anærobic bacteria fail to grow in the presence of air, not because atmospheric oxygen as such is a direct poison to the cell, but because of the toxic action of peroxides which may be formed as the result of the union of molecular oxygen

with some autoxidizable substance in the bacterial cell. Under these circumstances organisms which are peculiarly sensitive to the action of these peroxides may not only fail to grow but actually die. If this assumption is correct, then the ærobic growth of obligate anærobes in the presence of plant tissue finds partial explanation at least in the fact that peroxides formed are rapidly broken up by the oxidases of the tissue. Under these cultural conditions, therefore, so far as the toxic action of peroxides is concerned, the sensitive cell is protected almost as effectually as though it were growing under anærobic conditions.

Since iron is known to exert an accelerating action upon certain cellular oxidations, and is commonly found in conjunction with the peroxidase of plant tissue, it seemed possible that this substance might function in the oxidative mechanism of the bacterial cell and in the destruction of toxic peroxides in a manner analogous to that of plant tissue. As ferrous sulfate is known to accelerate many oxidation and reduction processes, and exhibits the usual reactions of peroxidase, iron in this form was chosen for study. However, when a solution of ferrous sulfate is added to broth, precipitation occurs. To overcome this, use was made of the method employed by Dony-Hennault in the preparation of artificial laccase. A solution of gum arabic and ferrous sulfate was precipitated in alcohol. The resulting precipitate is soluble in water and in aqueous solution gives the reaction of peroxidase with benzidine and hydrogen peroxide. Solutions of this gum-iron preparation remain stable in bouillon, the gum apparently functioning as a protective colloid. Quantitative analysis of this preparation shows that it contains approximately 20 mg. of iron per gram. In broth containing small amounts of this preparation the obligate anærobes studied were found to grow through repeated transfers in the presence of air.