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Factors influencing anaërobiosis with special reference to the use of fresh tissue.

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With methylene blue as an indicator, we have studied the influence of certain elements in promoting or in hindering the development of anaerobic conditions in tissue cultures.

As a result of our experiments, we have come to the following conclusions:

Liquid paraffin oil, used extensively as a seal for anaërobic cultures and in gas analysis, has very little value in inhibiting the access of oxygen. Solid vaseline, on the other hand, forms an effective oxygen-resisting seal. The difference is due to the physical states of the substances at incubator temperature.

Fresh kidney tissue is an active reducing agent and quickly decolorizes methylene blue in its vicinity. The reducing effect of fresh kidney tissue is relative to the amount used. As a reducing agent, at least 0.6 gm. per tube is required for the establishment of an adequate oxygen free zone.

Culture media may be classified as reducing or non-reducing. Those containing dextrose or peptone in a faintly alkaline solution belong to the former class. Ascitic fluid and dilute serum belong to the latter class, for their content of reducing substances is practically insignificant. For the prompt establishment of strictly anaërobic conditions these media require the addition of reducing substances such as dextrose, peptone, or kidney tissue aided by an effective seal or an anaërobic jar.

Semisolid media effectively inhibit the penetration of oxygen to the depths of the tube, but they likewise limit the diffusion of reducing substances and presumably of nutrient substances from imbedded kidney tissue.

The length of the column of medium is of minor importance under a vaseline seal.