

Oxygen Requirement of Pathogenic Bacteria Under Starving Conditions.

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The following experiments deal with quantitative changes in the nutrient content of the same kind of media, exposing the bacteria to slight or extensive starvation; but in all these media the bacteria could reproduce continuously, even if they showed morphological changes.

In shake-cultures using agar with decreasing amounts of nutrient broth, *B. coli* will grow as follows: with 100%-50% broth there is anaerobic and aerobic growth; with 20% there is growth only on the surface in a 2 mm thick layer; with 20%-5% broth the superficial layer becomes less dense and gradually deeper, reaching a thickness of 4 mm; with 3% there is no more growth on the surface; below the surface the colonies are very small, becoming gradually larger down to a depth of 5 mm. With 2%-0.1% broth there is no surface growth, but a free zone without any growth below the surface; the bacterial growth forms only a fine, $\frac{1}{4}$ - $\frac{1}{2}$ mm thick, dense, line of colonies 3-10 mm below the surface. The poorer the medium, the deeper lies the bacterial layer. The interspace between the surface and the zone of colonies does not show any visible growth. Bacteria do not die there, they are kept alive for several days.

This phenomenon may be more or less general. It was observed in cultures of aerobes like *B. prodigiosus* and *B. pyocyaneus* and in facultative anaerobes like *B. coli*, *B. typhosus*, *B. paratyphosus* B., *B. dysenteriae* Shiga and Flexner, *B. diphtheriae* and *Staphylococcus pyogenes aureus* and *albus*, and in pathogenic organisms as yeasts and actinomyces. These different groups may vary only in the amount of nutritive substance required to produce this phenomenon. Starving pneumococci, enterococci and as far as studied some streptococci do not show this growth in layers, probably because their respiratory system is different.

This zone of bacterial growth formed in shake-cultures under starving conditions is parallel to the surface and follows irregularities of the surface. Air bubbles placed in thin tubes filled with 1.25%

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agar containing 0.9% nutrient broth show concentric circles around the bubbles formed by colonies after 24 hours' incubation. An isolated colony of fungi located between the surface and a layer of *B. paratyphosus*, may cause an upward directed deviation of the latter layer; probably because the oxygen is partly used up by the fungi.

When the partial tension of oxygen above the medium is increased, the free zone without growth below the surface increases, and the layer of bacterial growth develops in a greater depth. Inversely, when the oxygen tension is decreased, growth appears on the surface in a medium which shows growth only in the depth, when exposed to air. In a medium just rich enough to show surface growth under atmospheric pressure, bacteria reproduce only below the surface, if exposed to increased oxygen tension.

"Bacterial zones" have been described previously by Beijerinck,¹ Jegunow,² Lehmann and Curchod,³ as a chemotactic phenomenon of motile organisms in liquid media. Eisenberg⁴ observed ring formation in shake-cultures of spore bearing bacteria in standard media. Braun⁵ is the first who mentions bacterial zones in gels of silicium-dioxyd with relation to nutrition, and recently Williams⁶ studied bacterial growth in long tubes under high pressure of different gases under different nutrition and obtained similar pictures using oxygen.

To find out which substance may be responsible for this phenomenon, *B. coli* were cultured in a synthetic medium. This medium contains only the indispensable substances for continuous growth for this group⁷: the mineral salts (K_2SO_4 , NaH_2PO_4 , Na_2HPO_4 , $MgSO_4$) and a single source of carbon (lactic acid) and a single source of nitrogen (NH_4Cl). In this type of media the bacteria are forced to a large extent to synthesize, in order to build up their body compounds; in this medium there is no anaerobic growth of facultative anaerobes.⁷ Shake-cultures using 1.25% agar containing gradually decreased quantities of this medium show a fine line of growth situated gradually deeper below the surface. When only the source of nitrogen is reduced and the source of carbon is kept constant, then this phenomenon does not appear. When the carbon compound which is also the main source of energy, is reduced, and the nitrogen compound is kept constant, then the characteristic structure of layer formation

¹ Beijerinck, M. W., *Cbl. f. Bakt.*, 1893, 14.

² Jegunow, *Cbl. f. Bakt.*, 1898, 2, 4.

³ Lehmann and Curchod, *Cbl. f. Bakt.*, 1905, 2, 14.

⁴ Eisenberg, *Cbl. f. Bakt. I*, 1919, 82, 209.

⁵ Braun, H., *Cbl. f. Bakt. I*, 1931, 122, 5.

⁶ Williams, J. W., *Growth*, 1939, 3, 21.

⁷ Braun, H., and Cahn-Bronner, C. E., *Cbl. f. Bakt.*, 1921, 86, 380; *Biochem. Z.*, 1922, 131, 272.

occurs.⁵ Thus the increased sensibility against oxygen is due to a lack of carbon compound, in other words, a lack of an easily oxidizable substance or an adequate oxygen acceptor. The less the medium contains of this substance, the more inhibitory is the action of oxygen on the organism, or may be the action of a substance formed by the bacteria in the presence of oxygen.

The distance of this thin layer of colonies from the surface varies with the temperature; it increases when the incubator-temperature of 37° is lowered to 22, 17, 12° or when it is raised above the optimal temperature of 37° to 42°. Exposure to sunlight also increases the free zone below the surface and the inhibitory action of oxygen; if a shake-culture is partially darkened by black paper, the bacterial layer develops closer to the surface in the darkened part, more distant from the surface in the exposed part of the agar column.

After some time of incubation under starving conditions 2, 3, sometimes up to 12 layers are formed below the uppermost layer which itself is distant from the surface. Each layer is separated from the one above by a narrow free zone. The number of these layers and the width of this formation is in relation to the amount of nutrient in the medium, the number of inoculated organism, and the temperature.

The unequal distribution of oxygen in the media is also an important factor in this ring-formation. The first layer forms by its respiration with extensive oxygen-intake a barrier against the diffusion of oxygen downwards. In the meantime the small quantity of nutrient which is present in the neighborhood of these colonies under starving conditions is partly used up, and inhibitory products are accumulated, thus a small free zone without growth develops beneath this layer and the next layer is formed after some time in a marked distance from the upper layer. This oxygen barrier of bacterial zones and the distribution of oxygen in colloidal media will be demonstrated in a subsequent report.

Conclusions and Summary. Under partial starvation some aerobic and facultative anaerobic bacteria, yeasts, and fungi grow only in a fine, dense layer below the surface of the shake-cultures where the relation between nutrient-content and oxygen is an optimum. Under increasing starvation bacteria become increasingly sensitive against oxygen. Oxygen causes a bacteriostatic effect, the rate of which is in relation to the nutrient content of the medium. The less the medium contains of carbon compounds which may act as oxygen acceptor, the more inhibitory is the action of oxygen on the organisms. Variation in temperature below and above the optimal temperature and exposure to light increases the sensibility against oxygen.