

of plasma phosphatase "is confined almost exclusively to cases of bone disease,"⁴ although Roberts had demonstrated increased plasma phosphatase in jaundice,⁶ and plasma or serum phosphatase has been stated to be almost exclusively of osseous origin.

It seems timely to point out the hepatogenous origin of some of the serum phosphatase as indicated by the high serum phosphatase found in jaundice (with the exception of some cases of anemia) and by the decrease of serum phosphatase in the course of successful treatment of catarrhal jaundice and hepatitis. The seeming exception of normal serum phosphatase figures in cases of jaundice with anemia confirms our view and emphasizes the hepatogenous rather than the hematogenous factor in jaundice as a source of increased phosphatase. It may be assumed, furthermore, that some of the serum phosphatase originates in the liver even under normal conditions.

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Factors Influencing Rate of Reduction of Potassium Ferricyanide by "Resting" *Escherichia coli*.

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It has been shown¹ that broth cultures of *Staphylococcus aureus* in the maximum stationary phase reduce potassium ferricyanide at a rate in the neighborhood of 6.6×10^{-14} millimols per cell per minute. Further work with cultures of *Escherichia coli* (K-12) gave similar results and will be reported elsewhere. The experiments to be reported here were designed to measure quantitatively the rate of reduction of potassium ferricyanide by "resting" *Esch. coli* under carefully controlled environmental conditions.

Forty-eight hour cultures of *Esch. coli*, grown on nutrient agar at 37.5° C., were suspended in saline and washed 3 times by centrifugation. The organisms were then diluted with a mixture of equal parts of M/15 phosphate buffer, pH 7.5, and physiological saline solution to a standard turbidity corresponding to a total count of approximately 20×10^8 cells per cubic centimeter.

⁶ Roberts, W. M., *Brit. J. Exp. Path.*, 1930, **11**, 90.

¹ Clifton, C. E., *J. Bact.*, 1933, **25**, 495.

Measured volumes of this suspension of "resting" *Esch coli* were introduced into tubes similar to those employed in the studies with *Staph. aureus*¹ and appropriate amounts of buffer, saline, and Difco peptone solutions were added. These suspensions were deaerated for one-half hour, by bubbling nitrogen through them at a constant rate, before the addition of a 0.1 molar solution of 3 parts of potassium ferricyanide to 1 part of potassium ferrocyanide. The change in the redox potential with time at 37.5°C. was followed potentiometrically at 10 minute intervals and the concentration of ferricyanide calculated directly from the potential readings.

The rate of reduction of the ferricyanide increases with increase in concentration of peptone (other variables being maintained constant) up to 2.0 or 3.0% and then decreases as the concentration of peptone is further increased. The rate of reduction of ferricyanide by "resting" *Esch. coli* in the absence of peptone or by peptone in the absence of "resting" bacteria is negligible. Duplicate tests agree within 1.0% and similar tests with suspensions prepared at different times give results of the same order of magnitude.

The number of millimols of ferricyanide reduced per cell per minute, in a 1% peptone solution, decreases as the concentration of bacteria is increased. Under the same conditions the rate of reduction of the ferricyanide increases as the concentration of the ferricyanide is increased. Similar results were obtained at pH 7.0 and pH 8.0. A decrease in temperature of 15°C. reduced the rate of reduction of the ferricyanide to approximately one-half of the value observed at 37.5°C.

The bearing of these results on the general problem of bacterial growth and behavior will be discussed in the final paper. On the basis of a considerable number of tests as summarized above we may, however, at present conclude that the concentration of peptone, organisms, and oxidant play important and closely connected rôles in controlling the metabolic activities of the cells in the average bacterial culture.