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**A comparative method for testing the enzymes of living hemolytic streptococci. I. Lipase.**

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Enzymes capable of digesting proteins, carbohydrates and fats have been demonstrated for the hemolytic streptococcus by Stevens and West who extracted these ferments from dead organisms by a process of grinding. This method is not available for quantitative determinations because of the difficulty of complete extraction by the grinding process. Nor can comparative determinations be made of different strains of the streptococci in culture media because of the variability of different strains in their rapidity of growth and because of the modifying factor of the media itself. It is possible, however, by the use of "indifferent" suspending fluids such as Locke's solution with 0.1 per cent gelatin or a solution containing 1.0 per cent sodium chloride and 0.05 per cent calcium chloride with 0.1 per cent gelatin and by quantitative determinations of bacterial concentration by means of the Gates turbidimeter, to compare the ferment action of a single organism under varying conditions or of different organisms under identical conditions.

The usual method of determining lipolytic activity by other workers has been to titrate with sodium hydroxide the acid produced in the enzyme substrate mixtures. Dietz has shown, however, that enzyme preparations of different activity, as measured by their velocity constants, reach the same equilibrium point if allowed to continue to completion. Within certain limits, when there is an excess of substrate present, the velocity of the reaction is in direct linear proportion to the quantity of active enzyme present. These facts together with the difficulty of titrating minute quantities of acid produced by small quantities of bacteria suggested the possibility of measuring the velocity of the reaction between certain definite hydrogen ion concentrations.

Standard color tubes were prepared by taking up heat killed organisms, in the same concentration as in the test suspensions, with Clark's buffer solutions at pH levels of 8.0, 7.6 and 7.2 with

phenol red as indicator. Then, under certain varying conditions, suspensions of living streptococci in the indifferent suspending fluids were allowed to act upon ethyl butyrate. In the active tubes the formation of butyric acid caused a more or less rapid change of color from pH 8.0 to 7.2. The rapidity of the change of color was taken as an indication of the activity of the lipolytic ferment under the given conditions.

With this method it was found that: (1) the acid production was more active in young cultures than in old; (2) there is an almost linear curve relationship between the speed of reaction and the concentration of the organisms; (3) the optimum temperature for the reaction is around 37.5°; (4) there is no action at 62° C. or above; (5) the property is destroyed by heating the bacteria to 60° C. or above for 10 minutes; (6) the optimum hydrogen ion concentration is around pH 7.8; (7) the activity is not increased by increasing the virulence of the organism by repeated rabbit passages; (8) the localization of the organism in the subcutaneous fat with a solution of this tissue in a certain clear cut clinical group of cases previously reported, is not due to a more active lipolytic ferment in the organisms recovered from these cases than is present in hemolytic streptococci from certain other clinically heterologous sources.

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### The relation between the roots of plants and fungi.

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The relation of the roots of plants to the fungi in the soil forming a true symbiosis, is of importance not only in botany from the light it throws on some forms of plant nutrition, but in medicine also from the close relation between symbiosis and parasitism.

The roots of all annual plants and many others are closely related to the soil surfaces by fine hollow processes given off from the epidermic cells termed root hairs. While the leaves of the plant through the chlorophyl are the agents of production of the