SCIENTIFIC PROCEEDINGS.

Western New York Branch.

Clifton Springs Sanitarium, December 11, 1926.

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The Inactivation of Crystallized Urease by Water and Its Prevention.

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Crystallized urease[1, 2] diluted with 100,000 times its weight of water, so that each cubic centimeter contains approximately one unit, is very rapidly inactivated at room temperature. The curve obtained when time is plotted against activity is practically a rectangular hyperbola. This inactivation by water is prevented more or less completely by amino acids, proteins, buffered cyanide, phosphates, and by many colloids other than proteins. Some of these colloids are: gum arabic, boiled starch, glycogen, aluminum hydroxide gel, gum mastic suspension and castor oil suspension. Glycocoll and alanin in 1 per cent solution do not afford complete protection, but neutral 2 per cent gum arabic protects perfectly. On account of this fact, 2 per cent gum arabic can be used to dilute crystallized urease when it is necessary to do so in order to test its activity. The protective action of phosphates is greater at pH 6.1 than at neutrality. Urease that has been largely inactivated by water can be partly reactivated by adding either gum arabic or amino acids. Urease is doubtless protected by urea, and also appears to protect itself. Urease crystals dissolved in only 1300 times their weight of water lose no more than 2 or 3 per cent of their activity during the first hour, as compared with a urease solution made by diluting the same quantity of crystals to the same volume, with 2 per cent gum arabic solution.

Impure urease solutions are largely protected by their impurities and show no measurable protective action by added amino acids and colloids when one unit of enzyme per cc. is tested for a period of 5 minutes. However, when diluted to contain only 0.1 unit per cc. and allowed to react with urea-phosphate for as long as 50 minutes at 20° C., impure urease plainly shows the protective action of amino acids and of colloids.

Rockwood[3, 4] and Rockwood and Husa[5] have maintained that the effect of amino acids on impure urease is largely a promoter instead of a protector one. Sherman and Walker[6, 7, 8] have disputed this, and claim that the effect is a protective one. My work with crystallized urease fails so far to show any promoter action, since the addition of amino acids or of colloids has never caused diluted urease to decompose urea more rapidly than an equal amount of undiluted urease.

The facts described in this paper show why urease is destroyed by dialysis. It is possible that many enzymes besides urease are capable of inactivation by water. The ability of urease to protect itself, when it has been not too greatly diluted, may be due to a combination of the electro-positive and electro-negative groups in one urease molecule with the electro-negative and electro-positive groups in another urease molecule. Amino acids possibly act in much the same manner, uniting with urease with both their amino and carboxyl groups. Colloids probably protect urease by condensing it upon their surfaces and thus preventing its dispersion.

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