Oscillatoria spread more extensively in isotope water, possibly due to the *P*H of 6.77 (kindly determined by Dr. T. L. Jahn with a glass electrode) for the difference was not as great in water samples buffered to 7.12.

The heavy water effects may be due to its influence on enzyme for a sample of pancreatic amylase in solution in the deuterium water for twenty-four hours was not as active in digesting starch as a similar portion in ordinary water (the erythrodextrin stage was reached in eight minutes in isotope water and in six minutes in controls). In this case it is suggested that the hydrolysis is retarded by the deuterium apart from a *P*H effect. Also, in fermentation tests a decrease of 10% in carbon dioxide production was observed when zymase was exposed for sixty-six hours to the isotope water.

Ice water may also condition enzyme action for Nord and Weiss [*Z. physik. Chem.*, **166**, 1 (1933)] report increased activity of enzyme solutions which have been frozen, possibly due to disaggregation of enzyme particles. Experiments by one of us (E. J. L.) in the Biology Department of Clark University show that oxidation of guaiaconic acid by the peroxidase–oxygenase system is increased if the solution is made up in freshly melted ice water and allowed to come to room temperature. When a solution of guaiaconic acid and peroxidase, without an oxygenase or peroxide, is frozen, oxidation occurs when the ice melts.

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THE ISOTOPIC ANALYSIS OF WATER

Sir:

The spectral method which was used in the original discovery of deuterium is not capable of high precision. The oxides of the two hydrogens, however, have considerably different physical properties which afford a basis for precise analytical methods. Densities have been used extensively,[1] and we have found that the determination of the index of refraction by means of a Zeiss interferometer is simple, rapid, and very precise. We feel that our present experience with this instrument may be of value to other investigators.

The actual measurement is a difference in index of refraction between ordinary water and water containing higher concentrations of deuterium oxide. The instrument was calibrated for direct refractive index measurements by potassium chloride solutions and for molal percentage of deuterium oxide by comparison with the corresponding densities. One

(1) Gilfillan and Polanyi have developed an ingenious method based on the principle of the Cartesian Diver, *Z. physik Chem.*, **166A**. 255 (1933).

division on the drum was found to be equal to 0.0106% of deuterium oxide. The drum readings were corrected for the familiar change in the fringe colors with drum setting. In the case of the 40-mm. cell it was found that the fringes are symmetrical at a reading of 55 divisions and again at intervals of 50 divisions. Thus from 55 to 105 the color match is made one fringe too high and the number of divisions (14) between the fringes must be subtracted from the reading.[2]

Samples of less than 20% concentration were measured with a 40-mm. cell. Higher concentrations can be handled by accurately diluting a small amount with ordinary water. Shorter cells such as the 10-mm. one with a 9-mm. inset giving a 1-mm. layer will handle the highest concentrations. The minimum quantity for the 40-mm. cell is 1.5 cc. and for the others the amount is correspondingly smaller. The precision of measurement is proportional to the length of cell, that for the 40-mm. chamber being about 0.02%. However, our present calibration for drum readings between 1500 and 3000 is not this precise.

Some precautions are necessary in preparing and handling the samples. A large bottle of redistilled water served as a standard. This was checked frequently by redistillation. Moderately concentrated samples for analysis were distilled in a small glass apparatus directly into the cell. Concentrated samples were distilled in a vacuum apparatus and drained into the cell through a tip on the end of the receiver. In the vacuum distillation the air can be removed by shaking during a preliminary freezing. Care must be observed to keep concentrated samples in water-free air to prevent serious dilution.

(2) See Gans and Bose, *Z. Instrumentenk.*, **36**, 137 (1916); L. H. Adams, THIS JOURNAL, **37**, 1181 (1915), for a discussion of this phenomenon.

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THE REFRACTIVE INDEX OF H_2O^{18}, AND THE COMPLETE ISOTOPIC ANALYSIS OF WATER

Sir:

In order to determine the validity of several additive laws in mixtures of H^1H^1O and H^2H^2O it has been necessary to dilute H^2H^2O with different amounts of ordinary water. The density of these mixtures and the refractive index, as well as the dependence of the latter upon temperature and wave length, have been determined. The complete results are contained in a paper which has been sent by one of us to the *Physical Review.*

The volumes proved to be not quite additive. When x, which is the mole fraction of H^2H^2O, or the atom fraction of H^2, is 0.50, the molal