[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF MINNESOTA]

Changes in *p*H of Irradiated Egg Albumin Solutions[1]

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A change can be observed in the *p*H of a protein solution after denaturation. The changes in *p*H produced by the denaturation process vary qualitatively and quantitatively, depending upon the type of denaturation employed. Previous reports in regard to the changes of *p*H resulting from ultraviolet denaturation of proteins in solutions are at variance. Mond[2] observed decreases of *p*H after ultraviolet irradiation of egg albumin and serum albumin solutions when the initial *p*H of the solution was above the protein isoelectric point, and a rise in *p*H when the initial *p*H was below the isoelectric point. Clark[3] found that the *p*H of egg albumin solutions decreased after irradiation when the initial *p*H was either acid or alkaline with respect to the isoelectric point. Stedman and Mendel[4] found that irradiation caused a drop in the *p*H, but the initial *p*H of the solutions was not given. Decreases in the *p*H of unbuffered distilled water after ultraviolet irradiation led these workers to interpret the drop in *p*H during irradiation of protein solutions as due to a change in the water. The present investigation was undertaken to examine the validity of these conflicting results.

Methods.—A Burdick air-cooled quartz mercury arc was used as a source of ultraviolet radiant energy. Two quartz mercury arcs, varying greatly in ultraviolet radiant energy output, were used during the investigation. Approximately 20 cc. of the egg albumin solutions were irradiated in quartz test-tubes (20 × 130 mm.), 19.5 cm. from the light source. The irradiation vessels were placed in a constant temperature water-bath held at 25°. Crystalline egg albumin was prepared by the method of Cole[5] and recrystallized twice Salts were removed, using the apparatus described by Bernhart, Arnow and Bratton.[6] About 10 cc. of a concentrated egg albumin solution, stored at 5° and preserved with a toluene paraffin oil layer, was measured accurately and diluted to 100 cc. with distilled water and a definite volume of hydrochloric acid or sodium hydroxide solution. The *p*H of this solution was measured with the glass electrode. Fresh solutions were prepared from the stock solution immediately before each irradiation. In one series of irradiations a nitrogen atmosphere was obtained by alternately evacuating and bubbling nitrogen through the solution (plus 0.02 cc. of *n*-heptyl alcohol to inhibit foaming) contained in the irradiation tube. The glass tubing employed was then sealed off and the stopper sealed. *n*-Heptyl alcohol (0.02 cc.) in 20 cc. of 0.01 *M* phosphate buffer (*p*H 6.5 were irradiated for forty-eight hours under nitrogen; this procedure caused no change in the *p*H of the mixture. In the other series of irradiations free access of air was obtained by fitting the stoppers of the quartz tubes with bent glass tubing. After each irradiation the *p*H of the solution was again determined.

Results

Change in *p*H as a Function of Initial *p*H.—The data obtained after forty-eight hours of irradiation are shown in Fig. 1. The results show

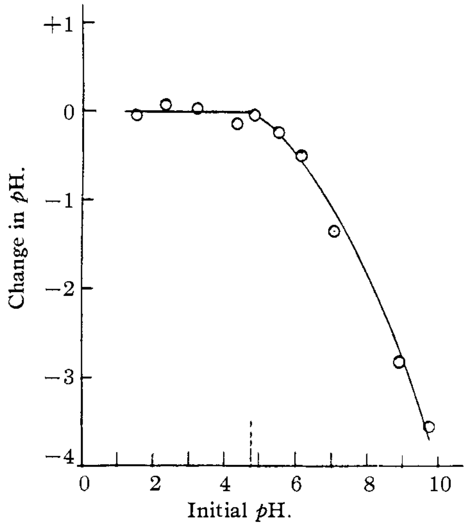

Fig. 1.—Change in *p*H of egg albumin solutions caused by forty-eight hours irradiation with arc 1W. 0.64 g. egg albumin per 100 cc. solutions irradiated in contact with air. The broken line, *p*H 4.8, indicates the isoelectric point of egg albumin.

that under the experimental conditions employed, a drop in *p*H occurs when the initial *p*H is above the isoelectric point of egg albumin (*p*H 4.8) and no significant change occurs when the initial *p*H is below the isoelectric point. A straight line relationship was found between the initial *p*H of the irradiated solution and the difference in base bound by egg albumin (Cohn[7]) at the *p*H values

(1) The data in this paper are taken from a thesis submitted by the author (present address: Department of Biochemistry, Tulane University, New Orleans, Louisiana) to the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(2) R. Mond, *Arch. Ges. Physiol.*, **200**, 374 (1923).

(3) J. H. Clark, *Am. J. Physiol.*, **61**, 72 (1922).

(4) H. L. Stedman and L. B. Mendel, *ibid.*, **77**, 199 (1926).

(5) A. G. Cole, *Proc. Soc. Exptl. Biol. Med.*, **30**, 1162 (1933).

(6) Bernhart, Arnow and Bratton, *Ind. Eng. Chem., Anal. Ed.*, **9**, 387 (1937).

(7) E. J. Cohn, *Physiol. Rev.*, **5**, 349 (1925).

before and after irradiation. This relationship is shown in Fig. 2. The difference in base bound

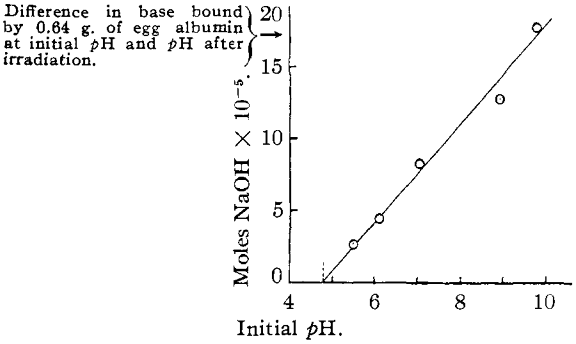

Fig. 2.—The difference in base bound by 0.64 g. egg albumin per 100 cc. at *p*H values before and after irradiation, as a function of the initial *p*H of the irradiated solutions. Experimental data same as Fig. 1.

by the egg albumin is a measure of the strong acid necessary to lower the *p*H to the same extent that it was lowered by irradiation. The data indicate that the photochemical reaction or reactions responsible for the drop in alkalinity of egg albumin solutions irradiated by ultraviolet light are catalyzed either directly or indirectly by the hydroxyl ion concentration of the solution. The change in *p*H observed is correlated with 90 to 98% denaturation of the egg albumin present. The extent of denaturation was determined by bringing the irradiated solution to the isoelectric point, then filtering, washing, drying, and weighing the precipitated protein.

Change in *p*H as a Function of the Time Irradiated.—The results obtained are shown in Fig. 3. The data show that after long periods of irradiation a slight rise in *p*H occurs in solutions whose initial *p*H is below the isoelectric point. Irradiation under nitrogen of a solution (*p*H 6.40) results in a rise of *p*H instead of the drop observed when the solutions are irradiated in air. This indicates that the reaction or reactions responsible for the decreases in *p*H require oxygen.

Discussion.—The data are in agreement with the work of Mond,[2] who concluded that the *p*H values of protein solutions tend to approach the isoelectric point under irradiation. The conclusion of Stedman and Mendel,[4] who believed that the drop in *p*H of irradiated protein solutions is due to a change in the solvent water, is untenable. Assuming the correctness of the methods by which

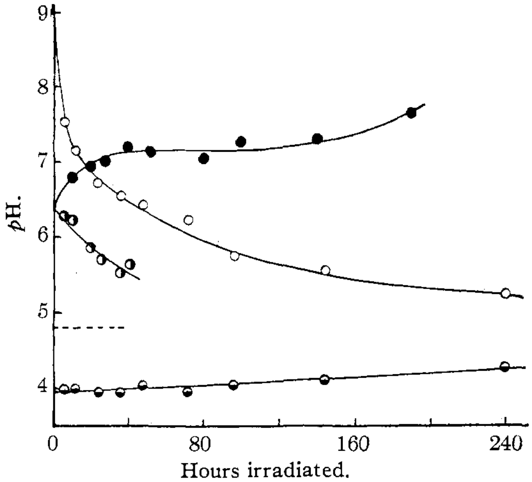

Fig. 3.—The change in *p*H of egg albumin solutions with the time irradiated: ○, 0.81 g. egg albumin per 100 cc., arc 1W, initial *p*H 9.03, irradiated in contact with air; ⊖, 0.81 g. egg albumin per 100 cc., arc 1W, initial *p*H 3.94, irradiated in contact with air; ●, 0.76 g. egg albumin per 100 cc., arc 2S, initial *p*H 6.40, irradiated under nitrogen; ◑, 0.76 g. egg albumin per 100 cc., arc 2S, initial *p*H 6.40, irradiated in contact with air.

decreases in *p*H in unbuffered distilled water were found, the extent of the change which they observed corresponds to a very small fraction of the acid formation necessary to reduce the *p*H of a protein–buffer solution. The largest *p*H change of distilled water resulting from ultraviolet irradiation, observed by Stedman and Mendel,[4] is from *p*H 7 to 5.8. The acid necessary to change the *p*H of 100 cc. of distilled water from *p*H 7 to 5.8 is approximately 0.14×10^{-5} mole of hydrochloric acid. In order to change the *p*H of 100 cc. of a solution containing 0.64 g. of egg albumin from *p*H 7 to 5.8, approximately 8×10^{-5} mole of hydrochloric acid is required.

Summary

The change in *p*H caused by ultraviolet irradiation of egg albumin solutions was studied with the glass electrode. The *p*H of egg albumin solutions irradiated in contact with air decreases when the *p*H is above the isoelectric point and rises slightly when the initial *p*H is below the isoelectric point. The *p*H of egg albumin solutions which are alkaline in respect to the isoelectric point increases during irradiation under nitrogen.

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