Study of the Color Reaction of Streptomycin Sulfate with Procaine Hydrochloride

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The cause of the increased color of streptomycin sulfate solution in the presence of procaine hydrochloride was studied. It was determined that the carbonyl function of the streptomycin reacted with the *p*-amino group of the procaine causing a discoloration beyond that of a normal streptomycin solution.

THE PURPOSE of this study was to investigate the cause of an increased darkening of streptomycin sulfate solution in the presence of procaine hydrochloride and to determine the functional groups responsible for this reaction.

This was of interest since procaine hydrochloride is frequently added to streptomycin sulfate solutions used for intramuscular or subcutaneous injection to alleviate pain at the injection site.

The streptomycin molecule is composed of three parts: streptidine, streptose, and N-methyl-L-glucosamine, connected to one another by glycosidic linkages (1). The presence of a reactive carbonyl group can be shown by its ability to undergo typical aldehyde reactions such as the formation of the oximes and semicarbazones (2). The preparation of biologically active N'-alkylstreptomycylamines have also been reported (3). Dihydrostreptomycin, due to the absence of this carbonyl group, is, therefore, a more stable, less reactive compound.

Streptomycin solution is subject to irreversible destruction by acid or alkali, and the rate of decomposition increases as the pH is lowered below 3.0 or elevated above 8.0. This decomposition is due to the hydrolysis of streptomycin producing streptidine and streptobiosamine (4). Streptomycin solutions normally darken upon standing. The extent and rate of color change depend in part on the purity of the preparation; however, this change in color is not necessarily accompanied by loss of activity.

EXPERIMENTAL

The streptomycin sulfate and dihydrostreptomycin sulfate used in this study were U.S.P. grade. The solutions used contained 400 mg. of antibiotic activity per ml. of an aqueous citrate solution, buffered at pH 6.0. The samples were stored at 50° in a constant temperature oven for various periods of time to accelerate reaction conditions. The absorbance was read on a Bausch and Lomb Spectronic 20 colorimeter at 450 mμ. The increase in absorbance under these conditions was a measure of the rate of color formation.

Solutions of streptomycin sulfate with procaine hydrochloride, tetracaine hydrochloride, and lidocaine hydrochloride in 2% concentrations were studied to determine their effect on the color formation of streptomycin in solution. Controls of the aforementioned compounds were also investigated. Figure 1 indicates that tetracaine hydrochloride and lidocaine hydrochloride had no effect on the color formation of streptomycin solutions. Procaine hydrochloride, however, greatly increased the color intensity of these solutions. This indicated that the primary amino group in the procaine hydrochloride molecule was the functional group involved

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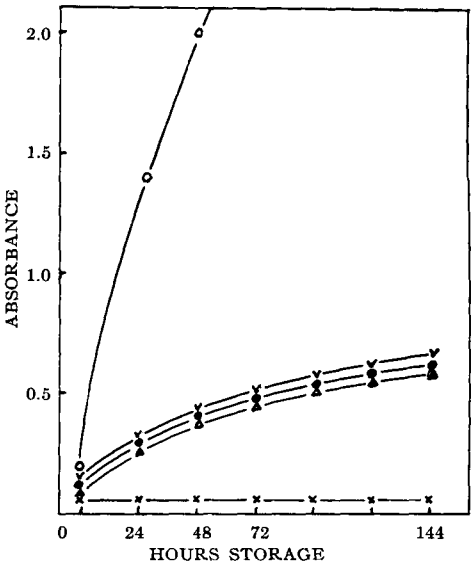

Fig. 1.—The effect of amines on streptomycin color formation. O, Streptomycin sulfate and 2% procaine hydrochloride; V, streptomycin sulfate and 2% tetracaine hydrochloride; ●, streptomycin sulfate; △, streptomycin sulfate and 2% lidocaine hydrochloride; x, 2% procaine hydrochloride, 2% tetracaine hydrochloride, and 2% lidocaine hydrochloride.

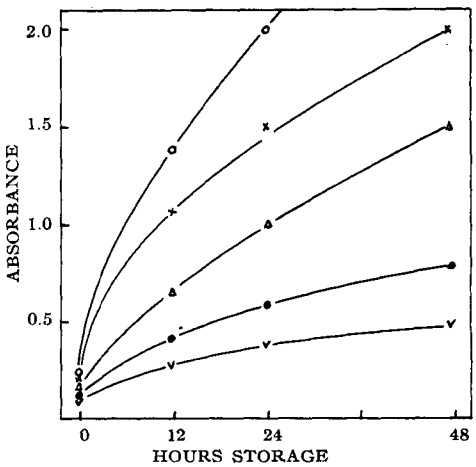

Fig. 2.—The effect of various concentrations of procaine hydrochloride on streptomycin color formation. V, Streptomycin solution; ●, streptomycin and 0.5% procaine hydrochloride; △, streptomycin and 1.0% procaine hydrochloride; x, streptomycin and 2.0% procaine hydrochloride; O, streptomycin and 4.0% procaine hydrochloride.

in this reaction. The effects of various concentrations of procaine hydrochloride on streptomycin solutions are shown in Fig. 2. The color of these solutions was intensified as the concentration of procaine hydrochloride was increased.

Since procaine hydrochloride upon hydrolysis forms *p*-aminobenzoic acid (5), solutions of streptomycin sulfate containing either 2% procaine hydrochloride or an equimolar quantity of *p*-aminobenzoic acid were comparatively evaluated. It has been reported that caffeine complexes with procaine (6) and *p*-aminobenzoic acid (7). Accordingly, the effect of caffeine on the color formation of these solutions was also investigated. The results are summarized in Fig. 3 and show that *p*-aminobenzoic acid has a more adverse effect upon the color stability of streptomycin solutions than does procaine hydrochloride. The addition of caffeine in no way affected the color formation of these solutions.

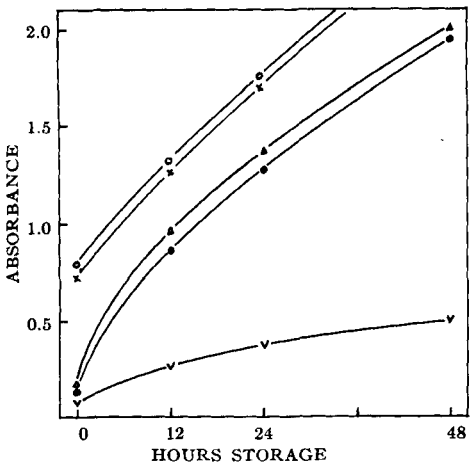

Fig. 3.—Effect of *p*-aminobenzoic acid, procaine hydrochloride, and caffeine on streptomycin color formation. ∨, Streptomycin; ●, streptomycin and 2% procaine hydrochloride; △, streptomycin, 2% procaine hydrochloride, and 2.0% caffeine; x, streptomycin and PABA; ○, streptomycin, PABA, and 2% caffeine.

To determine whether the carbonyl group of streptomycin was involved in this color reaction, dihydrostreptomycin was studied, since in this compound the carbonyl group is reduced to an alcohol. Upon the addition of procaine hydrochloride, tetracaine hydrochloride, or *p*-aminobenzoic acid to solutions of dihydrostreptomycin, no color was formed under accelerated conditions, thereby indicating that the carbonyl group of streptomycin was the active color-forming moiety involved in this reaction.

SUMMARY AND CONCLUSIONS

A study of the color reaction between procaine hydrochloride and streptomycin sulfate has shown that the darkening of this mixture beyond the normal colorless to light yellow color of a streptomycin solution was due to an interaction between the *p*-amino group of the procaine and the carbonyl group of the streptomycin. If the *p*-amino group was substituted as in the case of tetracaine hydrochloride, or absent as in lidocaine hydrochloride, or the carbonyl group reduced as in dihydrostreptomycin, this color formation did not occur under the conditions of this experiment.

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Book Notices

Instrumental Methods for the Analysis of Food Additives. Edited by William H. Butz and Henry J. Noebels. Interscience Publishers Inc., 250 Fifth Ave., New York 1, N. Y., 1961. viii + 288 pp. 15 × 23 cm. Price $11.

Timely and useful information is presented in this book. The text is divided into four major parts under the headings: Introduction, Sampling and cleanup, Identification, and Analytical procedures. The introduction is a 41-page coverage of the Food Additive Petition and discussions of the Food Additive Amendment and how to operate with it. An index is included.

The Fire of Life. By Max Kleiber. John Wiley & Sons, Inc., 440 Park Ave. South, New York 16, N. Y., 1961. xxii + 454 pp. 15 × 22.5 cm. Price $11.50.

An introductory textbook on animal energetics, this book aims to present the fundamental concepts (i.e., heat, latent heat, chemical energy, etc.) and their basic relationships. The book title indicates that the text is essentially limited to the classical rather than the newer aspect of metabolism and nutrition. The text is divided into six parts under the headings: Evolution of bioenergetics, Total starvation, Physical aspect of metabolism, Metabolism of the starving animal, Food as fuel, Food and population. Practice problems and an index are appended.

Carbon-14 Compounds. By John R. Catch. Butterworth Inc., 7235 Wisconsin Ave., Washington 14, D. C., 1961. vii + 128 pp. 14 × 21.5 cm.

Intended as a guide to the literature and not as a practical textbook nor a comprehensive index of C^{14}-compounds, this book touches on: The production of C^{14}, Chemical synthesis, Biological methods of labeling, Peculiar features of C^{14}-compounds, Analysis, Measurement of C^{14}, and Precautions in the use of C^{14}-compounds.