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— *The Editor comments* —

TO FOSTER CONTACT AND
STIMULATE RESEARCH

One of American pharmacy's greatest assets is its corps of distinguished scientists. While many outstanding pharmaceutical scientists are affiliated with various industrial laboratories, we wish to refer here particularly to those pharmaceutical scientists of note who are in other fields of endeavor. The majority in this group of course are associated on a full-time basis with educational institutions.

Almost all of these individuals are specialists in a single branch of the pharmaceutical sciences. Furthermore, from a geographic standpoint, they are scattered rather broadly across the country, and few institutions can boast of a faculty having more than one or two of these recognized scientists. Consequently, few students or even faculty members have had an opportunity to benefit from hearing or having contact with such outstanding authorities—except for the one or two who might be affiliated with their particular school.

This past year, however, has witnessed the initiation of a new program by the American Association of Colleges of Pharmacy with the primary objective of promoting education and research by fostering added opportunities for contact between productive and creative scientists and the staffs and students of our 74 pharmacy schools. This activity is described as the *Visiting Scientists Program* and is supported, in part, through a grant to the AACP from the National Science Foundation.

During this initial year of operation, 50 distinguished pharmaceutical authorities were nominated and accepted appointments to serve as visiting scientists. Some 77 visits totaling 119 days of visitation were arranged in order to bring these scientists to 45 of the 48 participating schools of pharmacy. By any measure employed, the program must be considered an outstanding success, and one which will have a continued beneficial effect on pharmaceutical research for many years to come. If there is any aspect of the program which might be criticized at all, it is that even more of the schools did not participate. Unfortunately, most of those which did not participate were schools most in need of the stimulating influence which a program of this nature might help to provide. Hopefully, however, we will see even greater participation if the program can be continued in future years.

Edward G. Feldmann

Stability of Procaine in Deuterium Oxide

By F. P. SIEGEL, F. D. HITER, S. V. SUSINA, and M. I. BLAKE

Stability of procaine in protium oxide and deuterium oxide was studied over the pH and "apparent" pH range 8 to 11. Studies were conducted at 40 and 100°.

PHARMACEUTICAL APPLICATIONS of the solvent deuterium oxide (heavy water) have not been explored. It appears from reports in the literature (1, 2) that deuterium oxide is toxic in animals only when the concentration in the body fluids reaches high levels. Since this solvent is now available in large amounts at a reasonable cost, and since deuterium oxide resembles protium oxide (ordinary water) more closely than any other solvent, it seems appropriate to study the effect of this solvent in pharmaceutical systems. Our present interest deals with the investigation of certain drugs which are labile in an aqueous medium. The stability of these drugs may be prolonged in deuterium oxide. This may prove of value, for example, in the extemporaneous preparation of ophthalmic solutions, where stability and sterility are important considerations. The effect of deuterium oxide in maintaining the sterility of such solutions is also under investigation in this laboratory.

In an earlier paper (3), the effect of deuterium oxide on the local anesthetic activity of procaine was studied. The cornea of the guinea pig was used as the test site. It was found that 0.5% solution of procaine in deuterium oxide gave the same protection as a 1.0% aqueous solution measured by the corneal reflex test.

The hydrolysis of procaine in aqueous solutions was studied by Higuchi, *et al.* (4). The rate of hydrolysis was shown to increase with hydroxide ion concentration. In the present study the rates of hydrolysis in protium and deuterium oxides are compared over the pH range 8.0 to 11.0. The effect of elevated temperature is also noted.

EXPERIMENTAL

The hydrolytic decomposition of procaine hydrochloride in protium oxide and deuterium oxide was followed by a modification of the method suggested by Higuchi, *et al.* (4). A series of ultraviolet absorption measurements was made on a solution of procaine hydrochloride undergoing hydrolysis in a constant temperature bath. Absorption measurements were made with a Beckman DU spectrophotometer model 2400 at wavelengths of 287 and 271.5 μ . The absorbance values obtained at the first wavelength corresponding to the absorption peak of procaine were used to calculate the per cent hydrolysis. The second wavelength readings are for the hydrolytic product, *p*-aminobenzoic acid, and serve as an internal check on side reactions which may occur. These readings should be constant since the absorption properties of procaine and *p*-aminobenzoic acid are identical at this wavelength. This has been noted by Higuchi, *et al.* Degree of hydrolysis was calculated by

$$\% \text{ ester remaining} = 100 \times \frac{(k_A - k_B)}{(k_C - k_B)} \quad (\text{Eq. 1})$$

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where k_A is the absorbance of a partially hydrolyzed 0.001% procaine hydrochloride solution, k_B is the absorbance of *p*-aminobenzoate ion equivalent to a 0.001% procaine hydrochloride solution, and k_C is the absorbance of an unhydrolyzed 0.001% procaine hydrochloride solution. All readings were taken at 287 μ .

The hydrolysis of procaine hydrochloride was studied over the pH range 8.0 to 11.0 in protium oxide and over the "apparent" pH range 8.0 to 11.0 in deuterium oxide. The hydroxide ion concentration was maintained essentially constant during the course of hydrolysis by buffering the solutions with 0.5 *M* ammonium hydroxide-ammonium chloride buffer.

The buffer solutions in deuterium oxide were prepared in the same manner as the aqueous buffers. The "apparent" pH was measured with a Beckman pH-meter (model H-2), and the solutions were adjusted to correspond to the aqueous pH values. An appropriate correction (5) to the "apparent" pH value was made to obtain pD. Analysis of the deuterium oxide buffers indicated that the deuterium oxide content was at least 99%.

A 50-ml. volumetric flask was filled to slightly below the mark with buffer solution and the flask was placed on a constant-temperature water bath set at $40 \pm 0.5^\circ$. When the solution in the flask reached the temperature of the bath, 50 λ of a freshly prepared 1.0% procaine hydrochloride stock solution was added. The flask was filled to the mark with buffer solution maintained at the temperature of the bath. The flask was then stoppered and shaken thoroughly. At zero time a 3.0-ml. sample was transferred from the flask to a spectrophotometer 1-cm. cell and the absorbance was determined. This corresponded to the k_C value of Eq. 1. The progress of hydrolysis was followed by removing samples in a similar manner at varying time intervals depending on the rate of hydrolysis. These readings gave k_A values for the above expression. Exactly 500 λ of a 0.503% *p*-aminobenzoic acid stock solution was added to a 5.0-ml. volumetric flask. The flask was filled to the mark with buffer solution and mixed well. Exactly 50 λ was transferred to a second 5.0-ml. volumetric flask. The flask was filled to the mark with buffer solution and shaken thoroughly. The absorbance was obtained and this supplied the k_B in the above expression. The concentration of *p*-aminobenzoate in the final dilution is equivalent to the concentration obtainable from a 0.001% procaine hydrochloride solution upon complete hydrolysis.

Since hydrolytic decomposition is accelerated at elevated temperatures, a comparative study was conducted at 100°. Exactly 50 λ of a 1.0% procaine hydrochloride solution was added to a 50-ml. volumetric flask. The flask was filled to the mark with pH 8.0 aqueous buffer or "apparent" pH 8.0 deuterium oxide buffer solution. The solution was mixed thoroughly and placed in 5.0-ml. colorbreak ampuls. The ampuls were sealed and placed in a boiling water bath. One ampul was set aside and was used to obtain the k_C reading. Ampuls were removed from the water bath at 5-minute intervals,

TABLE I.—COMPARISON OF RATE OF HYDROLYSIS OF PROCAINE IN PROTIUM OXIDE AND DEUTERIUM OXIDE AT 40°

Protium Oxide		Deuterium Oxide		$T_{1/2} \text{ D}_2\text{O}^a$ $T_{1/2} \text{ H}_2\text{O}$
pH	Half-life, hr.	pD	Half-life, hr.	
8.0	38.0	8.4 ^b	115.0	3.0
8.5	13.0	8.9	38.0	2.9
9.0	6.5	9.4	13.0	2.0
9.5	4.5	9.9	9.5	2.1
10.0	3.5	10.4	6.25	1.8
11.0	2.25	11.4	2.75	1.2
8.0 ^c	14 ^d	8.4	7.0 ^d	2.0

^a Ratio of half-lives at "apparent" pH in D₂O and pH in H₂O. ^b pD = pH + 0.4. ^c This run at 100° C. ^d Time for this run is in minutes.

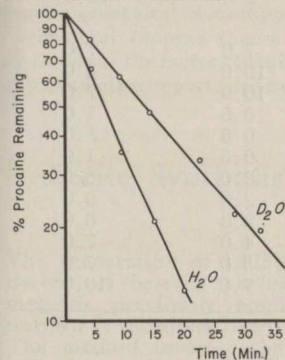


Fig. 1.—Comparison of rate of hydrolysis of procaine in protium oxide and deuterium oxide at pH 8.0 and at a 100° temperature.

plunged into an ice water bath, and the absorbance was obtained at 287 mμ. These supplied k_A readings. The k_B was obtained in the manner described for the 40° run.

The data obtained from the experiments described above are recorded in Table I. The hydrolysis rate of procaine at 100°, and pH 8 in protium oxide and "apparent" pH 8 in deuterium oxide is shown graphically in Fig. 1.

DISCUSSION

Over the pH range studied, the rate of deuteriolytic of procaine is less than the rate of hydrolysis. Over the pH and "apparent" pH range 8 to 11 and at 40° the ratio of half-lives in D₂O and H₂O is greatest at pH 8 (3.0) and decreases to a value of 1.2 at pH 11. Considerably higher ratios are obtained when the data at the equivalent pH and pD are compared. For example, when procaine in H₂O at pH 9.0 is compared with procaine in D₂O at pD 8.9, the ratio of half-lives is 5.8. However, when aqueous procaine at pH 9.0 is compared with procaine in D₂O at "apparent" pH 9.0, the ratio is 2.0, as shown in Table I. This would indicate that increased stability in D₂O is not simply a pH effect.

At 100° and pH 8 procaine is twice as stable in D₂O as in H₂O. The kinetic data for this study are shown graphically in Fig. 1. A typical first-order plot is obtained.

It was shown earlier (3) that the anesthetic activity of procaine, as observed on the cornea of the guinea pig, was greater in D₂O by a factor of 2. Since the anesthetic activity of procaine is due solely to free base which is predominant in the alkaline pH range, increased activity is, therefore, attributed to a greater stability (*in vivo*) of procaine base in deuterium oxide. Further, at the same pH and "apparent" pH, a deuterium oxide solution of procaine may contain more free base than in the corresponding aqueous solution.

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Effects of Limbic Lesions on Chlorpromazine-Pentobarbital Interaction

By MARVIN COHEN* and JOHN W. NELSON

Small lesions within the limbic system of the rat produced no consistent behavioral defects or altered responses to chlorpromazine and/or pentobarbital measured by a new behavioral scoring method.

THE LIMBIC SYSTEM is a portion of the brain located on the medial and basal walls of the cerebral hemispheres. Numerous stimulation and ablation studies (1-5) have indicated that this system is involved in the production or modification of emotional (affective) behavior. Other studies of a similar type have suggested that the psychotropic drug chlorpromazine may exert some of its behavioral effects through a mechanism involving the limbic system (6, 7). However, no portion of this system has definitely been shown to be a site for chlorpromazine. An investigation was undertaken

to determine if chlorpromazine did act within the limbic system. This was done by the examination of responses to chlorpromazine after small lesions had been placed in selected areas of the limbic system.

EXPERIMENTAL

The animals used were albino male rats of the Wistar strain and 70-100 days old. All animals were kept in groups of four and allowed free access to food and water, except during testing and operating periods. Lesions were made electrolytically with a Grass lesion maker (model LM-1). The current passed into each brain was of sufficient intensity to produce a lesion 0.5 to 1 mm. in diameter. All lesions were verified histologically by conventional paraffin embedding procedures. Sections were stained with a combination of Luxol fast blue, hematoxylin, and eosin to obtain maximum differentiation of the lesion. Sham operations were performed by anesthetizing an animal, trephining its skull, and inserting the needle electrode to the appropriate brain area without allowing electric cur-

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* Present address: IIT Research Institute, Chicago, Ill.