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

Protium Oxide pH	Protium Oxide Half-life, hr.	Deuterium Oxide pD	Deuterium Oxide Half-life, hr.	$T_{1/2}$ D_2O / $T_{1/2}$ H_2O [a]
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 D_2O and pH in H_2O. [b] pD = pH + 0.4. [c] This run at 100° C. [d] Time for this run is in minutes.

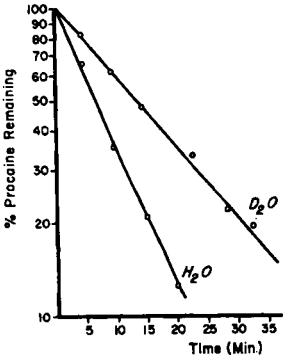

Fig. 1.—Comparison of rate of hydrolysis of procaine in protium oxide and deuterium oxide at pH and "apparent" pH 8.0 and at a 100° temperature.

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 deuteriolysis 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 D_2O and H_2O 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 H_2O at pH 9.0 is compared with procaine in D_2O 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 D_2O at "apparent" pH 9.0, the ratio is 2.0, as shown in Table I. This would indicate that increased stability in D_2O is not simply a pH effect.

At 100° and pH 8 procaine is twice as stable in D_2O as in H_2O. 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 D_2O 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.

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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-

TABLE I.—RESPONSES OF ANIMALS WITH SEPTAL LESIONS

Animal No.	Response to Saline	Effect of Chlorpromazine Dose	Effect of Chlorpromazine Response, mg./Kg.	Effect of Pentobarbital Dose	Effect of Pentobarbital Response, mg./Kg.	Response to Combination
			Unoperated Animals			
13	0.5	1	1.0	17.5	10.0	7.0
31	1.0	1	2.0	17.5	7.0	7.0
47	2.0	1	3.0	17.5	10.0	10.0
14	1.5	3	4.0	5	1.5	1.0
39	2.0	3	3.0	5	2.0	4.0
55	0.5	3	0.5	5	0.5	0.5
15	2.5	5	5.0	10	1.0	6.0
45	3.0	5	5.0	10	3.0	4.0
74	1.0	5	3.0	10	2.0	6.0
16	1.0	7	3.0	15	7.0	6.0
53	0.5	7	4.0	15	3.0	7.0
76	1.5	7	5.0	15	2.0	10.0
			Operated Animals			
13[a]	0.5	1	0.5	17.5	7.0	7.0
31	0.5	1	1.0	17.5	10.0	3.0
47	2.0	1	1.0	17.5	10.0	7.0
14[a]	1.0	3	1.0	5	0.5	1.0
39	1.0	3	1.0	5	0.5	1.0
55	0.5	3	1.0	5	0.5	1.0
15[a]	1.0	5	3.0	10	3.0	6.0
45	1.0	5	5.0	10	2.0	5.0
74	1.0	5	4.0	10	2.0	6.0
16[a]	1.0	7	4.0	15	4.0	7.0
53	0.5	7	4.0	15	3.0	7.0
76	1.0	7	5.0	15	7.0	10.0

[a] Sham operation.

TABLE II.—RESPONSES OF ANIMALS WITH AMYGDALOID LESIONS

Animal No.	Response to Saline	Effect of Chlorpromazine Dose	Effect of Chlorpromazine Response, mg./Kg.	Effect of Pentobarbital Dose	Effect of Pentobarbital Response, mg./Kg.	Response to Combination
			Unoperated Animals			
75	3.0	1	2.0	17.5	10.0	7.0
73	1.0	3	4.0	5	2.5	3.0
33	1.0	3	1.0	5	1.0	2.0
46	3.0	3	3.0	5	3.0	4.0
80	0.5	5	5.0	10	2.0	6.0
21	1.0	5	5.0	10	1.0	5.0
34	1.0	5	1.0	10	3.0	4.0
78	3.0	7	4.5	15	6.0	10.0
30	1.0	7	5.0	15	3.0	5.0
36	1.0	7	5.0	15	6.0	6.0
			Operated Animals			
75[a]	1.0	1	1.0	17.5	10.0	10.0
73[a]	1.0	3	3.0	5	0.5	3.0
33	0.5	3	1.0	5	0.5	2.0
46	2.5	3	2.0	5	2.0	2.0
80[a]	1.0	5	3.0	10	1.0	4.0
21	1.0	5	3.0	10	1.0	5.0
34	1.0	5	4.0	10	3.0	5.0
78[a]	0.0	7	4.0	15	5.0	10.0
36	1.0	7	4.0	15	3.0	10.0
30	0.5	7	1.0	15	3.0	7.0

[a] Sham operation.

rent to pass. The wound was sutured, and the animal was allowed to recover.

Behavioral changes were determined by means of an original method which evaluated the responses to low (subhypnotic) doses of central depressants with a scoring system. The behavioral criteria utilized as the basis of the scoring system were loss of spontaneous motion, responses to external stimuli, and degree of ataxia. This method has been described in detail in a previous publication (8).

RESULTS

Four areas of the limbic system—the anterior (precommissural) septum, amygdaloid complex, rostral hippocampus, and medial forebrain bundle—were chosen for study. Unoperated and operated animals were tested for their responses to pentobarbital (5, 10, 15, or 17.5 mg./Kg.), chlorpromazine (1, 3, 5, or 7 mg./Kg.), and combinations of the two (3 mg./Kg. chlorpromazine + 5 mg./Kg. pentobarbital, 5 mg./Kg. chlorpromazine + 10 mg./Kg. pentobarbital, 7 mg./Kg. chlorpromazine + 15 mg./Kg. pentobarbital, or 1 mg./Kg. chlorpromazine + 17.5 mg./Kg. pentobarbital). All drugs were administered intraperitoneally. Combinations of chlorpromazine and pentobarbital were used because they gave a more sensitive indication of the behavioral effects of chlorpromazine than did the

latter administered alone, using the criteria of the behavioral method employed.

Tables I and II show the responses obtained with animals subjected to septal and amygdaloid lesions, respectively. It can be seen that small lesions in these areas produced no consistent change in behavior or in the responses to the administration of the drugs used. Similar results were obtained with the other areas investigated. The only behavioral change noted was a slight hyperexcitability; but since sham operated animals also showed this reaction, it was attributed to the operating procedure rather than to the presence of a lesion.

The results of this study indicate that investigations of behavior involving the production of lesions require a great deal of caution in the interpretation of behavioral changes that are seen. Lesion size may be one of the factors that has contributed to the many conflicting reports appearing in this field.

SUMMARY

Small lesions within the limbic system of the rat produced no consistent behavioral defects or altered responses to chlorpromazine and/or pentobarbital as measured by a new behavioral scoring method. Lesion size may be one of the factors that has contributed to the many conflicting reports appearing in this field.

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Facile Synthesis of Isoindoline and Substituted Isoindolines

By JOHN L. NEUMEYER*

The preparation of isoindoline and several derivatives bearing nuclear substituents by methods previously employed successfully only for *N*-substituted derivatives is described. The method involves the preparation and hydrogenolysis of *N*-benzyl isoindolines.

WHILE A considerable amount of work has been reported on the synthesis of *N*-substituted isoindolines and hydrogenated isoindolines by a variety of methods, difficulty was experienced by several workers (including the author) in obtaining isoindoline (VI*a*) in good yields and in a pure form. Isoindoline derivatives have been obtained by the reaction of amines with *o*-xylylene dibromide (1, 2). Bornstein (3) prepared isoindoline (VI*a*) by the hydrolytic cleavage of 2-(*p*-tolylsulfonyl)-isoindoline (prepared from *o*-xylylene dibromide and *p*-toluenesulfonamide) with phenol and hydrobromic acid in propionic acid. The reduction of *N*-substituted phthalimides with lithium aluminum hydride was a generally satisfactory method for obtaining *N*-substituted isoindolines (4, 5) and was utilized also for the preparation of tetrahydro and hexahydroisondoline (4), but failed when applied to the reduction of phthalimide. As a result, we have devised a method for the preparation of isoindoline and substituted isoindolines which was based on the lithium aluminum hydride reduction of *N*-benzylphthalimides of their corresponding isoindolines, followed by the hydrogenolysis of the benzyl moiety with palladium-on-charcoal. This route was satisfactory also for the preparation of 5-methylisoindoline (VI*b*) and 4-aminoisoindoline (VI*d*), but failed to yield 4-chloroisoindoline from the hydrogenolysis of *N*-benzyl-4-chloroisoindoline (V*c*). Treatment of V*c* with hydrogen and palladium caused the hydrogenolysis of the chlorine and the benzyl group; isoindoline (VI*a*) was the isolated product.

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EXPERIMENTAL[1]

N-Benzylphthalimide.—This was prepared from potassium phthalimide (0.5 mole) and benzyl chloride (0.5 mole) in *N*,*N*-dimethylformamide in 75% yield by the method of Billman and Cash (6), crude, m.p. 111–112°. The compound was used in subsequent reactions without further purification. [Lit. (6) m.p. 115–116°.]

N-Benzyl-4-methylphthalimide (IIb).—This was obtained from potassium 4-methylphthalimide (I*b*) and benzyl chloride; yield: 72%, m.p. 122.5 to 123.5°.

Anal.—Calcd. for $C_{16}H_{13}NO_2$: C, 73.99; H, 5.77; N, 5.57. Found: C, 73.87; H, 5.82; N, 5.63.

N-Benzyl-3-chlorophthalimide (IIc).—This was obtained from potassium 3-chlorophthalimide (I*c*) and benzyl chloride; yield: 98%, m.p. 137 to 138.5° (recrystallized from *n*-butanol).

Anal.—Calcd. for $C_{15}H_{10}NO_2Cl$: C, 66.30; H, 3.71. Found: C, 66.29; H, 3.71.

N-Benzyl-3-nitrophthalimide (IId).—A mixture of 19.3 Gm. (0.1 mole) of 3-nitrophthalic anhydride and 10.7 Gm. (0.1 mole) of benzylamine was fused at 200–250° and the mixture allowed to cool. Upon recrystallization from isopropyl alcohol, 23 Gm. (83%) of yellow flakes, m.p. 140–141°, were obtained.

Anal.—Calcd. for $C_{15}H_{10}N_2O_4$: C, 63.83; H, 3.57; N, 9.93. Found: C, 63.68; H, 3.74; N, 9.90.

N-Benzyl-3-aminophthalimide (IIe).—A solution of 25 Gm. (0.089 mole) of *N*-benzyl-3-nitrophthalimide in 290 ml. of benzene was hydrogenated in a Parr hydrogenator at room temperature using 0.5 Gm. of platinum oxide until the hydrogen uptake

[1] Both the melting points and the boiling points were uncorrected. All melting points were obtained in a Thomas Hoover silicone oil filled capillary melting point apparatus. The assistance of Mr. R. C. Pharo with a number of the experiments reported herein, of Mr. J. E. Zarembo and his staff in carrying out the analyses, and of Mr. H. Adelman in the interpretation of infrared spectra is gratefully acknowledged.