with *A. niger* when evaluating the effect of methylcellulose and polyethylene glycol 6000 on PCMX; and the result obtained with *Ps. aeruginosa* in evaluating the effect of polysorbate 80 at a concentration of 0.5% on PCMX. In these instances, no increase was observed. In all cases where a reduction in the antimicrobial activity of PCMX was demonstrated, the reduction was thought to be a direct result of the molecular interaction with the nonionic macromolecule, thereby diminishing the availability of the drug to exert its antimicrobial activity.

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*Keyphrases*

*p*-Chloro-*m*-xylenol (PCMX)—microbiological evaluation
Minimum inhibitory concentration—PCMX
Complex formation—PCMX activity
Equilibrium dialysis—PCMX, methylcellulose, polysorbate 80
Spectrophotometry—cell count determinations


# Studies on the Effects of Reserpine in Mice as Influenced by its Diluent

By W. VELDKAMP, G. A. JOHNSON, and H. H. KEASLING

**The effects of dextroamphetamine sulfate and benzphetamine hydrochloride on locomotor activity in mice pretreated with reserpine varied depending on the reserpine preparations used. Pretreatment with reserpine suspended in aqueous 0.25 percent methylcellulose (pH 5.8) had little effect on the response to dextroamphetamine sulfate but decreased the response to benzphetamine hydrochloride. Pretreatment with a reserpine solution (pH 3.5) significantly enhanced the locomotor response to both compounds at the higher doses tested. The difference in effect produced by the reserpine preparations could not be explained on the basis of differences in brain amine levels at the time locomotor activity was measured since both preparations had depleted brain amines to the same extent. Further studies revealed that variations in pH of the reserpine diluent had a marked effect on brain reserpine levels, on brain amine levels when measured 1 hr. after reserpine administration, and on the rate of onset and intensity of ptosis produced in the mouse.**

VAN ROSSUM *et al.* (1) and van Rossum and Hurkmans (2) have reported that reserpine pretreatment in mice has no effect on the locomotor activity response to dextroamphetamine sulfate while the effects of derivatives of amphetamine which have large substituents on the nitrogen atom, such as benzylamphetamine and benzphetamine hydrochloride,[1] are antagonized by pretreatment with reserpine. On the other hand, Smith (3) has reported an enhancement of the effects of dextroamphetamine sulfate on the locomotor activity of mice when pretreated with reserpine.

The present study shows that the effect obtained with dextroamphetamine sulfate and benzphetamine hydrochloride in reserpinized mice will vary depending upon the reserpine preparation used in pretreating the mice. A number of acids such as ascorbic, Burn and Rand, (4), acetic, Fleming and Trendelenberg, (5), and citric, Fleming, (6) have been used to dissolve reser-

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[1] Trademarked as Didrex by the Upjohn Co., Kalamazoo, Mich.

pine in preparation for parenteral administration. In this study the results obtained when mice were pretreated with a reserpine preparation containing citric acid were compared with results from mice pretreated with reserpine suspended in aqueous 0.25% methylcellulose. The following parameters were measured in making these comparisons: locomotor activity after administration of dextroamphetamine sulfate or benzphetamine hydrochloride in the reserpinized mouse and the effects of pH of the reserpine diluent on the onset and intensity of ptosis, on the depletion of biogenic amines, and on brain levels of reserpine.

## METHODS

Carworth Farms No. 1 male albino mice weighing between 20 and 25 Gm. were used. Each mouse was used only once.

**Measurement of Locomotor Activity**—Eight Metro Industries[2] circular actophotometers, each equipped with 6 light beams and 6 photoelectric cells connected to a digital counter, were used. A single mouse was placed in each actophotometer and the number of times the mouse crossed a beam of light was recorded. In determining the locomotor activity effects of dextroamphetamine sulfate and benzphetamine hydrochloride, groups of 8 mice were injected with various doses of the compounds and immediately placed in individual actophotometers. After a 10-min. acclimation period, locomotor activity was recorded for a 30-min. period. Each experiment was carried out using an 8 × 8 Latin square design balanced with respect to treatments, actophotometers and time of day.

**Drugs**—The reserpine solution used in the motor activity studies contained the following per ml. of solution: 0.25 mg. recrystallized reserpine, 0.20 mg. citric acid anhydrous, 9.0 mg. benzyl alcohol, 15.0 mg. *N,N*-dimethylacetamide, water, q.s., to make 1 ml. This solution had a pH of approximately 3.5. The reserpine suspension contained 0.25 mg. reserpine per ml. of aqueous 0.25% methylcellulose and had a pH of approximately 5.8. In the experiments where the effects of pH were studied, the stock reserpine solution was diluted with 0.25 volume of either distilled water or an 0.25% aqueous methylcellulose solution. The pH was adjusted to the desired pH with 0.1 *N* HCl or 0.5 *M* $NaHCO_3$. The final concentration of reserpine was 0.2 mg./ml. Dextroamphetamine sulfate and benzphetamine hydrochloride were dissolved in saline and the dosages expressed in terms of the salts. All injections were made intraperitoneally.

**Reserpine Assay**—Two mouse brains were pooled for each determination. Tissues were homogenized and reserpine was extracted by the methods reported by Hess *et al.* (7). The assay of reserpine was modified from that of Hess *et al.* by the omission of selenious acid from the assay mixture. The quantitative determination of reserpine was based upon the fluorescence of reserpine (0.2 mcg.) in duplicate internal standards in split brain homogenates. Fluorescence of reserpine in this assay was linear over the range 0.01–0.2 mcg.

[2] Metro Industries, Mineola, L.I., N.Y.

**Biogenic Amine Assays**—The extraction procedure of Shore and Olin (8) was employed for the extraction of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) from paired mouse brains. Aliquots of the resulting 4-ml. aqueous extract were removed for the respective analyses with the noted modifications: NE, Shore and Olin (8); DA, Carlsson and Waldeck (9), with reduced volumes to increase the sensitivity and a final acid addition of 5 *N* acetic acid–5 *N* HCl, 2:3; 5-HT, Snyder *et al.* (10), with heating at 60° for 1 hr. All calculations of amine content were based upon tissue-containing internal standards and are expressed as mcg./Gm. wet weight of brain tissue. Results were not corrected for recovery of amines by the extraction procedure.

**Ptosis Scoring**—Ptosis was graded on a 4-point scale (1 = normal, 4 = complete ptosis) in groups of six mice, 1, 2, 3, and 18 hr. after intraperitoneal administration of reserpine, Rubin *et al.* (11).

## RESULTS

**Locomotor Activity in Mice Pretreated with Either a Reserpine Solution or a Reserpine Suspension**—The effects of dextroamphetamine sulfate and benzphetamine hydrochloride on the 30-min. locomotor activity of mice pretreated once daily for 3 days with either saline or 1 mg./Kg. of reserpine suspension with motor activity run on the fourth day are shown in Fig. 1. Pretreatment with the reserpine suspension decreased the effect of benzphetamine hydrochloride on locomotor activity of both doses tested. Dextroamphetamine sulfate in similarly pretreated mice was less effective at the 0.625 mg./Kg. dose while the 2.5 mg./Kg. dose caused a slight but nonsignificant increase in motor activity compared to the effect in saline pretreated controls.

When the mice were pretreated with the reserpine solution, there was no quantitative difference between the effects of dextroamphetamine sulfate and benzphetamine hydrochloride. Figure 2 shows the results obtained with mice pretreated once daily for 3 days with 1 mg./Kg. of reserpine solution and 30-min. locomotor activity run on the fourth day. At the lower dose both dextroamphetamine sulfate and benzphetamine hydrochloride were less stimulant, while at the higher dose both caused a significantly greater increase in locomotor activity than in the saline pretreated controls.

Brain amine levels after 3 days pretreatment do not explain the difference in results obtained with the two reserpine preparations on motor activity with dextroamphetamine sulfate and benzphetamine hydrochloride. Groups of mice were injected once daily for 3 days with 1 mg./Kg. of either the reserpine solution or suspension and brain amine levels were determined on the fourth day. Results in Table I show that there was no significant difference between the reserpine preparations in their effect on brain amine levels under these conditions.

**Effects of pH on Reserpine Activity**—Since there was a difference in the pH of the reserpine solution (pH 3.5) and the reserpine suspension (pH 5.8) used in the above locomotor activity studies, the effects of pH on reserpine activity were investigated.

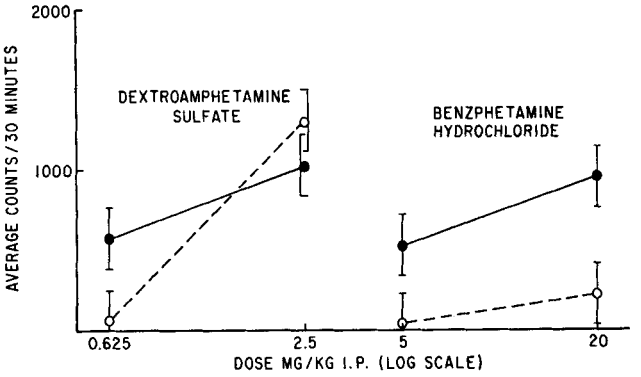


*Fig. 1—Effects of reserpine suspension (1 mg./Kg./day, 3 days) on locomotor activity response of mice to dextroamphetamine sulfate and benzphetamine hydrochloride. Key: I, represents ± S.E. of mean; O—O, reserpine suspension pretreatment; ●——●, saline, pretreatment.*

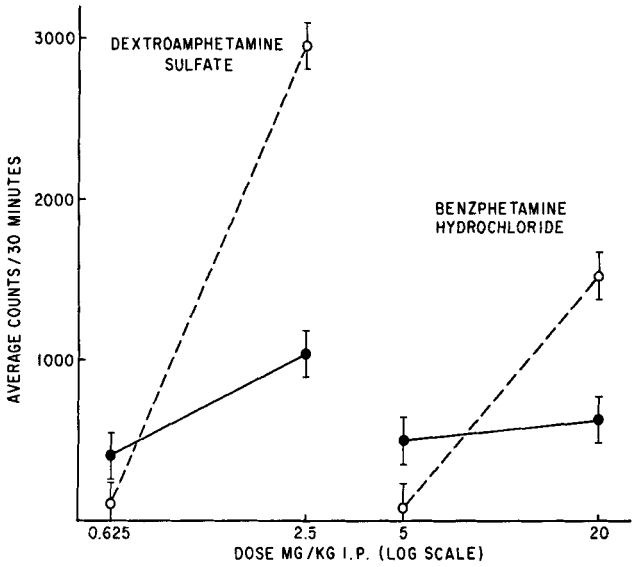


*Fig. 2—Effect of reserpine solution (1 mg./Kg./day, 3 days) on locomotor activity response of mice to dextroamphetamine sulfate and benzphetamine hydrochloride. Key: I, represents ± S.E. of mean; O—O, reserpine solution, pretreatment; ●——●, saline, pretreatment.*

TABLE I—BRAIN NOREPINEPHRINE AND SEROTONIN LEVELS IN MICE AFTER 1 mg./Kg. RESERPINE ONCE DAILY FOR THREE DAYS

| | Norepinephrine | | | Serotonin | | |
|---|---|---|---|---|---|---|
| Preparation | No. of Determinations | Concentration mcg./Gm. ± S.E.M. | % of Control | No. of Determinations | Concentration mcg./Gm. ± S.E.M. | % of Control |
| Saline control | 5 | 0.50 ± 0.04 | 100 | 5 | 0.80 ± 0.07 | 100 |
| Reserpine solution | 4 | 0.05 ± 0.01 | 10 | 4 | 0.51 ± 0.04 | 64 |
| Reserpine suspension | 3 | 0.05 ± 0.01 | 10 | 3 | 0.46 ± 0.06 | 60 |

Effect of diluent pH on brain levels of reserpine at various intervals after its intraperitoneal administration is shown in Fig. 3. Maximal reserpine levels were detected with the pH 2.8, 3.5, and 4.5 solutions in the 10–20 min. interval after injection. Reserpine was not detected in the brain following intraperitoneal administration of the pH 5.7 preparation and only trace quantities were found with the pH 2.0 solution. Brain levels of reserpine after intraperitoneal administration of reserpine suspended in aqueous 0.25% methylcellulose at pH 3.7 are less than those obtained after the pH 3.5 reserpine solution.

Variations in diluent pH of the reserpine preparations also influenced the depletion of endogenous brain amines. The effects of diluent pH on the depletion of brain amines during the first hour after intraperitoneal administration of reserpine (5 mg./Kg.) are shown in Table II. The most marked depletion of norepinephrine, dopamine, and serotonin was noted with the reserpine preparation at pH 2.8. No decrease in amine content at 1 hr. was detected with the pH 5.7 and pH 7.0 preparations.

The onset and intensity of ptosis induced by reserpine (2 mg./Kg., i.p.) as influenced by the pH of its diluent is shown in Fig. 4. The reserpine preparation with pH 3.5 caused the greatest degree of ptosis. With all the preparations except the one at pH 7.0 maximum ptosis was detected at the 3-hr. reading. A moderate amount of ptosis was present at 18 hr. with the pH 7.0 preparation.

## DISCUSSION

The results presented indicate that the effect of dextroamphetamine sulfate and benzphetamine hydrochloride on locomotor activity in reserpinized mice varies depending on the reserpine preparation

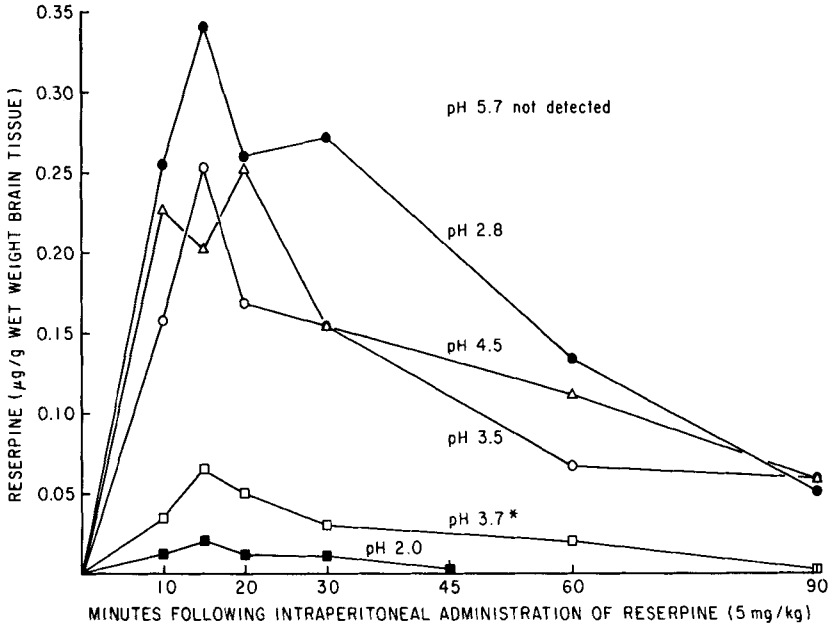


*Fig. 3—Effect of diluent pH on brain reserpine content. Stock reserpine solution was diluted with 0.25 volume of distilled water and the pH adjusted as described in* Methods. *At pH 5.7 no reserpine was detected. * The reserpine for these results was suspended in 0.25% aqueous methylcellulose and pH adjusted to 3.7.*

TABLE II—EFFECT OF DILUENT pH ON ENDOGENOUS MOUSE BRAIN AMINE LEVELS BY RESERPINE (5 mg./Kg., i.p.) AFTER ONE HOUR[a]

| Diluent pH | NE | DA | 5-HT |
|---|---|---|---|
| 2.0 | 0.44 ± 0.14[b] (4)[c] | 0.46 ± 0.27 (4) | 0.69 ± 0.17 (4) |
| 2.8 | 0.24 ± 0.04 (4) | 0.23 ± 0.08 (4) | 0.52 ± 0.05 (4) |
| 3.5 | 0.31 ± 0.03 (4) | 0.36 ± 0.14 (4) | 0.56 ± 0.05 (4) |
| 4.5 | 0.37 ± 0.17 (4) | 0.51 ± 0.44 (4) | 0.57 ± 0.17 (4) |
| 5.7 | 0.51 ± 0.09 (6) | 0.82 ± 0.13 (4) | 0.88 ± 0.06 (6) |
| 7.0 | 0.52 ± 0.13 (6) | 0.85 ± 0.17 (4) | 0.90 ± 0.07 (6) |

[a] Brain amine levels from control animals in these five experiments were: NE, 0.52 ± 0.04 (7); DA, 0.83 ± 0.05 (4); 5-HT, 0.89 ± 0.07 (7). [b] All values are expressed as mg. amine/Gm. wet weight brain tissue ± S.D. [c] Number of determinations.

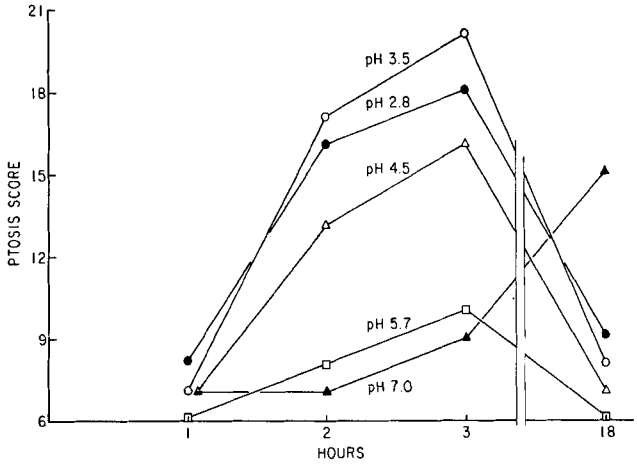


*Fig. 4—Effect of diluent pH on reserpine (2 mg./Kg., i.p.) induced ptosis. Composition of the reserpine preparation is the same as described for stock reserpine solution in Fig. 3.*

used in pretreating the mice. Pretreatment of mice with a reserpine suspension (pH 5.8) resulted in a decreased motor response to both the low (2.5 mg./Kg.) and high (20 mg./Kg.) dose of benzphetamine hydrochloride. The response to the low (0.625 mg./Kg.) dose of dextroamphetamine sulfate was depressed while there was a slight but non-significant increase at the high (2.5 mg./Kg.) dose. Mice pretreated with the reserpine solution (pH 3.5) had a decreased locomotor activity response to the low dose of both dextroamphetamine sulfate and benzphetamine hydrochloride but the high dose of both compounds caused a significant increase in activity.

The results presented here with dextroamphetamine sulfate in mice pretreated with reserpine solution are in agreement with those reported by Smith (3) who used reserpine solubilized with ascorbic acid to pretreat his mice. The results obtained with both dextroamphetamine sulfate and benzphetamine hydrochloride in mice pretreated with the reserpine solution do not agree, however, with those of van Rossum *et al.* (1, 2) who used acetic acid to solubilize the reserpine (personal communication). Only when the mice were pretreated with a reserpine suspension could the results reported by van Rossum *et al.* be repeated.

Brain amine levels after pretreatment with either the reserpine solution or suspension for 3 days at 1 mg./Kg./day do not explain the differences in results obtained on locomotor activity. These preparations both depleted measurable brain amine levels about the same extent.

The results shown in Fig. 3 indicate that whether the reserpine is in solution or suspension and the pH of the diluent influence the initial rate of reserpine uptake and the level of reserpine attained in the brain. This in turn is thought to influence the rate of decline in the brain amine levels. The pH of the reserpine solution used to pretreat the mice for the locomotor activity studies was 3.5 and therefore gave higher and more rapid reserpine levels in the brain and more rapid decline of brain amine than did the reserpine suspension which had a pH of 5.8. If the reasoning of Stein (12) is correct that reserpine pretreatment may cause supersensitivity to amines in the brain as well as in the periphery, and if the sensitivity is increased with time then the increased locomotor response in the mice pretreated with the reserpine solution may be due to the increased length of time these mice had to develop the supersensitivity.

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*Keyphrases*

Reserpine activity—diluent effect
Methylcellulose—reserpine activity
Citric acid solution—reserpine activity
Dextroamphetamine $SO_4$ activity—reserpine–diluent effect
Benzphetamine HCl activity—reserpine–diluent effect
Brain, mouse—analysis
Locomotor activity—analysis

# Potassium Absorption—A Comparison of *In Vitro* and *In Vivo* Studies

By A. J. JOUHAR, E. S. GARNETT, and J. S. WALLINGTON

**Some $^{42}K$ was released from a slow release preparation within 15 min. of ingestion and further potassium release continued for approximately 200 min. A similar absorption pattern was found from enteric-coated tablets of potassium chloride although a considerable initial delay occurred before potassium was released from this preparation. The *in vivo* behavior of the slow release preparation and enteric-coated tablets was similar to that seen in static solution experiments but differed considerably from that found in the B.P. disintegration apparatus. There was no evidence that sudden release and absorption of potassium took place from enteric-coated tablets.**

LONG CONTINUED ADMINISTRATION of thiazide diuretics reduces exchangeable potassium, and the need for potassium supplements is well recognized (1, 2). These may be given separately or in a compound tablet, such as the film-coated, two-layered slow release preparation.[1] In this preparation crystals of potassium chloride (KCl) are coated with a polymeric material and compressed to form one layer, and the other layer contains bendrofluazide. The KCl layer is intended to dissolve gradually and so release potassium ions ($K^+$) continuously. Since it has been

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[1] Tradename of Neo-NaClex-K.