

The Pharmacology of Reserpine in the Horse. II. Effects of Reserpine on Platelet Serotonin Uptake and Behavior

T. Tobin, D.V.M., Ph.D.
C.G. White, M.Sc.
W.E. Woods, M.Sc.

From the Kentucky Equine Drug Research Program, Department of Veterinary Science, College of Agriculture, University of Kentucky, and the Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40546.

Publication No. 42 from the Kentucky Equine Drug Research and Testing Programs, Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, KY 40546. Published as Kentucky Agricultural Experiment Station Article No. 78-4-188 with the permission of the Dean and Director, College of Agriculture and Agricultural Experiment Station.

The assistance of Jerry Voos and Edith Nugent, who helped with the counting procedure, is gratefully acknowledged. Supported by grants from The United States Trotting Association, the Kentucky Equine Research Fund, The Patricia Hewitt Foundation, and a U.K.R.F. Graduate Fellowship.

The effect of reserpine administered *in vivo* on the *in vitro* uptake of [^3H] serotonin by horse platelets was studied. Inhibition by reserpine of platelet serotonin uptake was biphasic, peaking at about 75% inhibition at two and eight hours post-drug administration and then returning to control values. Essentially maximal inhibition of platelet serotonin uptake was seen at all doses of reserpine above 0.1 mg/1000 lb, while little inhibition was seen at 0.05 mg/1000 lb. The experiments show a very steep dose-response curve for reserpine inhibition of serotonin uptake, with a midpoint slope of >2.6 . Because significant behavioral and clinical signs of reserpine treatment were not seen at doses of less than 1 mg/1000 lb, blockade of platelet serotonin uptake by reserpine is a very sensitive indicator of treatment with reserpine.

Introduction

Reserpine is the principal alkaloid found in the roots of *Rauwolfia serpentina*, a plant indigenous to India and Southeast Asia.⁶ It is a potent tranquilizer and is reportedly used to "take the edge off" hyperexcitable race, trotting and show horses.⁷ Reserpine apparently produces its principal pharmacological effects by interfering with catecholamine and/or serotonin metabolism, and in a previous paper the effects of reserpine on equine platelet serotonin uptake *in vitro* are characterized.¹⁰

In this paper we further characterize the uptake of serotonin by equine platelets. Platelet uptake of serotonin is very sensitive to reserpine *in vivo*, and doses of reserpine as low as 0.1 mg/1000 lb intravenous (IV) inhibit platelet uptake of serotonin for more than 12 hours. No behavioral effects or clinical signs of treatment with reserpine were seen after this dose. Platelet serotonin uptake is thus a readily measurable index of reserpine pretreatment in horses, and is highly sensitive to the presence of this drug.

Materials and Methods

Mature Thoroughbred mares and geldings between 400 and 500 kg body weight were used throughout. All animals were housed in individual loose boxes and fed hay, oats and water *ad libitum*. All drug administration and sample taking were done in these loose boxes. All drug administration was by rapid intravenous (IV) injection, and all blood samples were drawn into vacutainer tubes^a containing Na₂ EDTA.

Platelet uptake of [³H] serotonin^b was determined as previously described,¹⁰ and expressed as μ moles serotonin/ 10^{11} platelets/minute. Because platelets rapidly lost their ability to take up [³H] serotonin during these experiments (Figure 1), the experiments were performed as soon as possible after drawing blood samples, and all uptake rates were calculated from data obtained within 20 minutes of starting the experiment.

Spontaneous locomotor activity of control and reserpine^c-treated horses was determined by tallying steps

^a Becton-Dickinson, Boston, MA.

^b New England Nuclear, Boston, MA. (Carrier serotonin was from the Sigma Chemical Company, St. Louis, MO.)

^c Serpasil[®], Ciba-Geigy Pharmaceutical Company, St. Louis, MO.

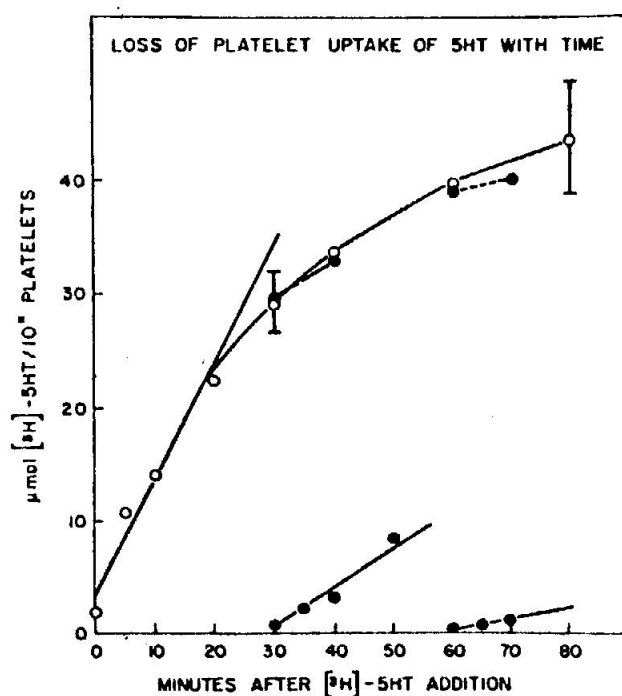


Figure 1. Loss of platelet uptake of [³H] serotonin with time. The open circles (O-O) show the rate of uptake of [³H] serotonin by horse platelets *in vitro* at 37°C. The solid circles (●-●) show uptake after preincubation of the platelets at 37°C for 30 and 60 minutes, respectively, before addition of [³H] serotonin. The slopes of the initial uptake rates from these preincubated samples, dashed lines (----), are also superimposed on the control curve for comparison.

with the left foreleg for a 30-minute period each morning as previously described.⁸ Since activity of a depressant drug was being measured, the absolute number of counts per minute was small, so counts for each 30-minute period were pooled and expressed as a percentage of counts for the control period.

Results

While it was initially assumed that the decrease in rate of serotonin uptake with time was a steady state phenomenon in a pump-leak system, observations on the rate of loss of platelet motility prompted us to test this hypothesis. Figure 1 shows that [³H] serotonin uptake by platelets declined at the same rate whether or not serotonin was present in the system. Because an initial uptake rate was therefore the only meaningful measure of platelet uptake activity, all experiments were limited to the first twenty minutes of uptake and an initial rate calculated from these values. This initial uptake rate was remarkably consistent from horse to horse as shown in Table 1. In other experiments,⁹ (data not presented) an apparent K_m for serotonin uptake by horse platelets of 2.04×10^{-7} M and a V_{max} of 5.0μ mol/ 10^{11} platelets/minute were determined from initial rate data.

Figure 2 shows the effect of 10 mg/1000 lb of reserpine administered *in vivo* on platelet uptake of [³H] serotonin *in vitro*. The solid circle shows the initial uptake rate of [³H] serotonin in these platelets normalized as 100%, with the rates of postreserpine expressed as a percentage of this value. Platelet uptake of serotonin

TABLE 1

Initial rates of platelet serotonin uptake
in 11 horses.

Horse	Initial Rate of Uptake (μ mole [³ H] 5-HT/ 10^{11} platelets/minute)
T-1	1.5
T-3	1.2
T-4	1.2
T-8	1.1
T-9	1.2
T-10	1.0
Devil Dora	1.3
Ballard's Love Bug	0.8
Winged Feather	0.85
Bea Lucky	1.5
Reggie	1.1
(X \pm SEM)	1.16 \pm 0.07

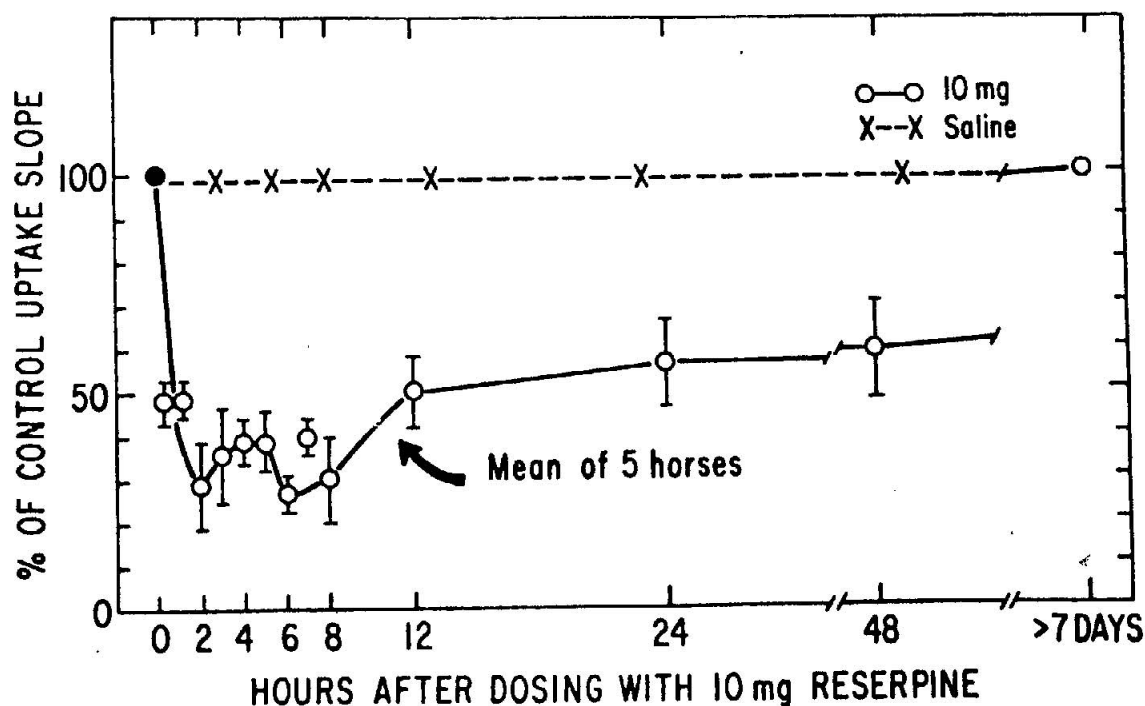


Figure 2. Inhibition of initial rate of uptake of $[^3\text{H}]$ serotonin by platelets *in vitro* after reserpine *in vivo*. The solid circle (●) represents the mean rate of $[^3\text{H}]$ serotonin uptake by platelets from five horses. The open circles (○—○) show the mean rate at the indicated times after dosing with 10 mg/100 lb reserpine. The dashed line (x—x) shows uptake by equine platelets after saline treatment. All points are the mean \pm SEM of experiments on at least four horses.

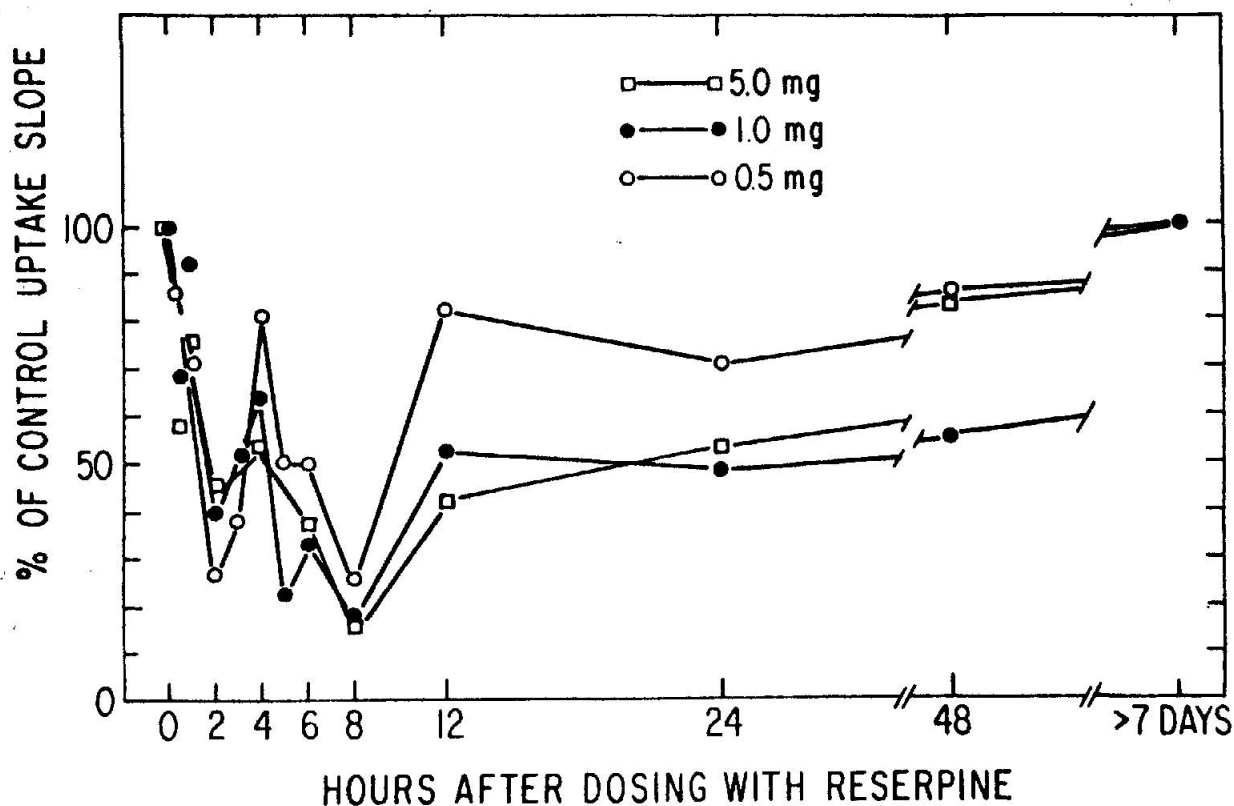


Figure 3. Inhibition of initial rate of uptake of $[^3\text{H}]$ serotonin by platelets after reserpine *in vivo*. The open squares (□—□) show platelet uptake of $[^3\text{H}]$ serotonin at the indicated times after 5.0 mg/1000 lb reserpine. The closed circles (●—●) represent platelet uptake after a 1.0 mg/1000 lb, while the open circles (○—○) represent uptake after 0.5 mg/1000 lb reserpine. All points represent experimental determinations in single horses.

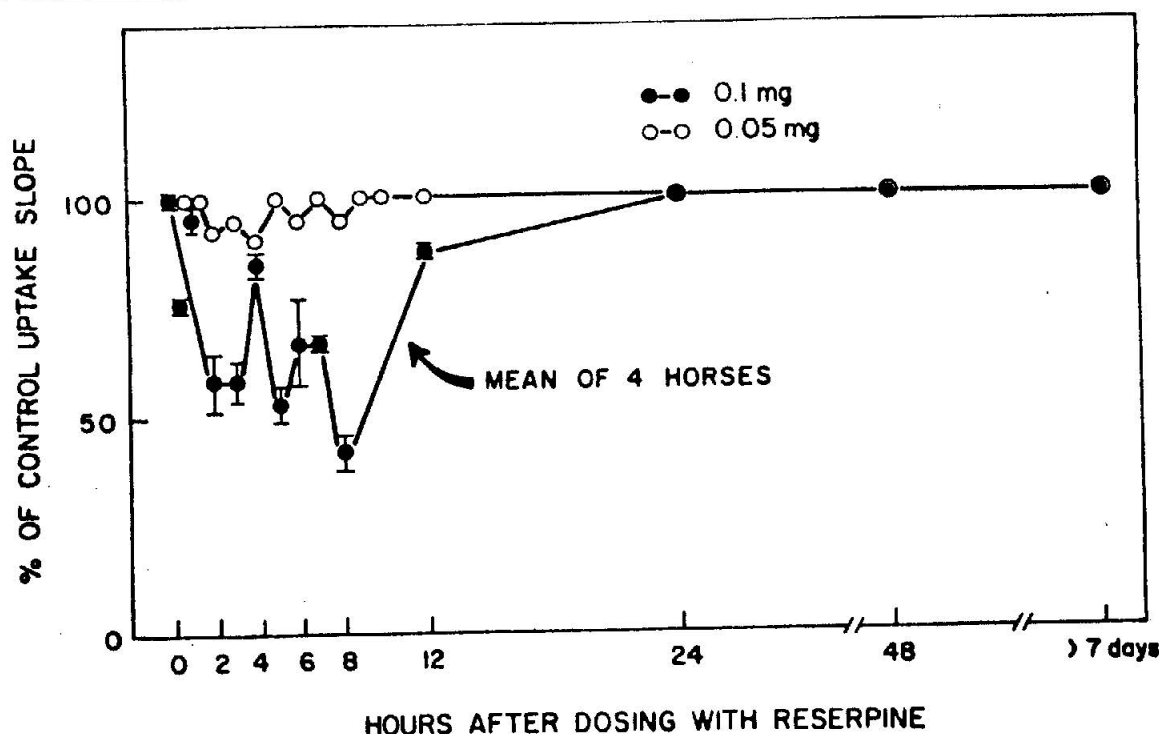


Figure 4. Inhibition of initial rate of uptake of [^3H] serotonin in equine platelets after reserpine *in vivo*. The solid circles (●-●) show mean platelet uptake of [^3H] serotonin \pm SEM after 0.1 mg/1000 lb of reserpine in four horses. The open circles (○-○) represent platelet uptake after 0.05 mg/1000 lb of reserpine in two horses.

dropped to 30% of control within two hours, remained depressed until eight hours postdosing and then slowly recovered toward control. Uptake was still 40% inhibited 48 hours after reserpine and required more than seven days postdosing to fully recover. No significant changes in the rate of [^3H] serotonin uptake by platelets from control horses were observed.

Figures 3 and 4 show the effects of smaller doses of reserpine on the initial rate of serotonin uptake by horse platelets. Results broadly similar to those observed at 10 mg/1000 lb were produced by doses of 5.0, 1.0 and 0.5 mg/1000 lb, respectively (Figure 3). At a dose of 0.1 mg/1000 lb (0.22 $\mu\text{g}/\text{kg}$), substantial inhibition of the initial uptake rate was still observed, but in this case the inhibition had almost reversed within 12 hours and had completely reversed by 24 hours. However, halving the dose to 0.05 mg/1000 lb produced essentially no effect on platelet serotonin uptake.

Figure 5 shows the inhibition observed at eight hours postreserpine for each dose plotted as a dose-response curve. The data are approximately fitted by a smooth curve and maximal inhibition was observed at the 0.5 mg/1000 lb dose of reserpine. The dose-response curve climbs steeply between 0.05 and 0.1 mg/1000 lb reserpine, with a midpoint slope of at least 2.6.

To quantitate the effects of reserpine on equine behavior, four horses were dosed with 12 mg/1000 lb of reserpine. Spontaneous motor activity in these animals was monitored by counting steps taken with the left fore-

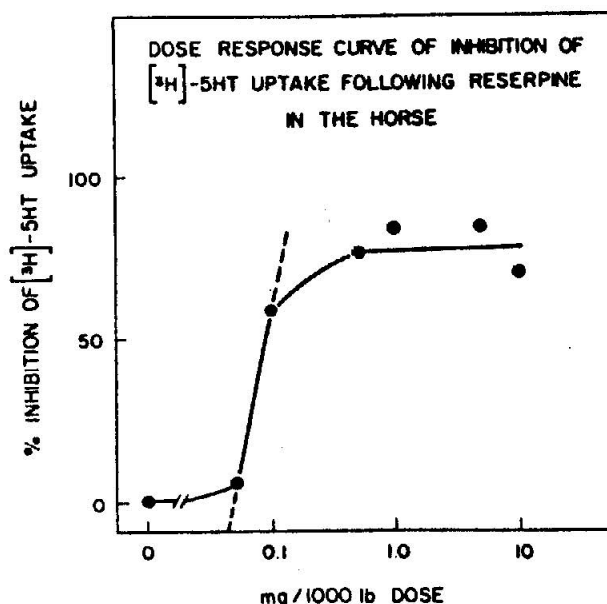


Figure 5. Dose-response curve for inhibition of [^3H] serotonin uptake following reserpine *in vivo*. The dose-response curve was generated by plotting dose of reserpine against the percent inhibition of [^3H] serotonin uptake eight hours after dosing.

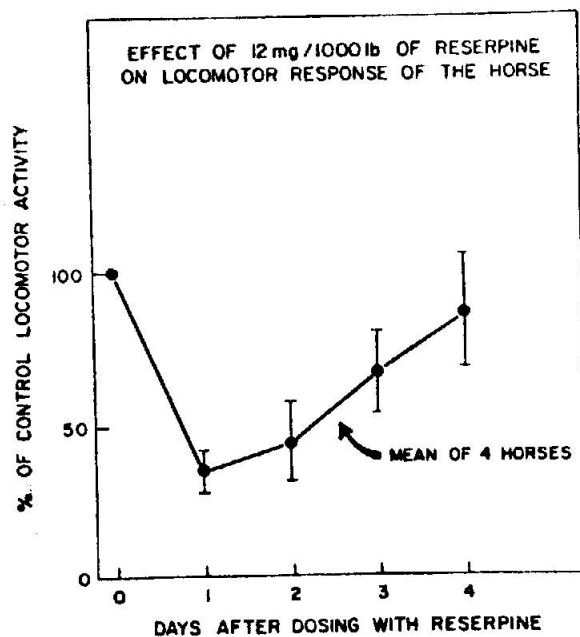


Figure 6. Effect of reserpine on spontaneous locomotor activity of the horse. The closed circles (●-●) represent the change in locomotor response from control for four horses (means \pm SEM) after a 12 mg/1000 lb dose of reserpine. Locomotor activity was determined as described in "Methods" and was significantly reduced ($P < 0.05$) for three days after dosing.

leg for a 30-minute period each morning. Figure 6 shows that spontaneous motor activity in these horses was inhibited about 60% on the first day postdosing, after which it slowly returned toward control. By four days postdosing there were no significant differences between the pre- and postreserpine locomotor activity values.

Throughout these experiments the animals were watched carefully for clinical signs of reserpinization. At 10 mg/1000 lb IV of reserpine, the first signs were sweating over the shoulder, back, stifle and inguinal regions three hours after dosing. At about this time passage of flatus increased, and signs of increased gastrointestinal motility and diarrhea appeared. In male animals penile extension started at about five hours, was maximal at 12 hours and was almost completely withdrawn by 24 hours. By eight hours postdosing, all animals dosed with 10 mg/1000 lb IV were standing immobile and depressed and some animals showed signs of colic and went down for a short period. By 72 hours postdosing all animals dosed with 10 mg/1000 lb IV appeared clinically normal.

Horses dosed with 5 mg/1000 lb IV reserpine exhibited ptosis at about three hours and gastrointestinal distress and diarrhea at eight hours. At 1 mg/1000 lb the signs were correspondingly reduced, and at 0.5 mg/1000 lb the horses appeared normal except for the passage of some very loose feces at about 10 hours. No clinical or behavioral signs of reserpinization were seen at lower doses of reserpine.

Discussion

Uptake of serotonin by horse platelets was a very sensitive indicator of reserpine administration to horses, substantial inhibition being observed after a dose of reserpine of 0.1 mg/1000 lb IV. Since minimal clinical signs of reserpine administration were seen at doses of less than 1 mg/1000 lb, it appears that serotonin uptake by horse platelets is about 10 times more sensitive an indicator of reserpinization than is behavior of the whole animal.

The inhibition of platelet [^3H] serotonin uptake was characteristically biphasic. Inhibition peaked first at about two hours postdosing, decreased by the fourth hour postdosing and then increased again to a maximum by eight hours postdosing. After the second peak, inhibition again declined, to stabilize at about 12 hours postdosing and remain at this level for at least 48 hours at the higher doses tested. At the 0.1 mg/1000 lb dose, platelet uptake of [^3H] serotonin had returned to control value within 24 hours. The rate of recovery of platelet uptake of [^3H] serotonin was not followed at the higher doses, except that horses were tested at more than seven days postdosing and found to have returned to control values.

The simplest explanation for the rather complex biphasic pattern of inhibition seen is that reserpine may have been rapidly excreted in the bile. Reabsorption from the gastrointestinal tract would then give rise to the second and greater peak of inhibition. While no reports of biliary excretion of reserpine exist, reserpine levels are rapidly reduced by passage through the liver in rats,⁶ and tritium-labeled compounds were detected in feces for 12 days after administration of [^3H] reserpine.⁴ Thus an enterohepatic circulation of reserpine, accounting for the biphasic peaks of inhibition of serotonin uptake, appears possible, although at this point no direct supporting evidence is available.

An unexpected finding in these experiments has been the apparent rapidity with which the platelets recovered from inhibition of serotonin uptake. Reserpine is generally considered an irreversibly binding drug,³ and once inhibition due to reserpine occurs, it is not thought to reverse.⁶ In sharp contrast with this concept, the individual data of Figures 2, 3 and 4 all showed patterns of rapid reversal of reserpine inhibition. This reversal was especially apparent at the lowest dose tested, where reversal started at eight hours, was almost complete by 12 hours and was complete at 24 hours. This rapid reversal, and the biphasic pattern of inhibition seen throughout these experiments, is not consistent with irreversible binding of reserpine at its receptor sites, but rather suggests a reversible interaction.

If the interaction of reserpine with its pharmacological receptors is reversible, then the reason for the pro-

longed inhibition observed at the higher doses must be related to plasma levels of the drug rather than to irreversibly bound reserpine. This possibility is supported by the high lipid solubility of reserpine,⁶ suggesting a large volume of distribution for this drug in the body and likely a long plasma half-life. Preliminary data on the pharmacokinetics of reserpine in the horse suggests that reserpine can be detected in equine plasma for at least five days,⁴ and reserpine has been reported to have a half-life of up to 11 days in humans.⁴ In view of this evidence of a very long plasma half-life for reserpine, there is no necessity to postulate irreversible binding of reserpine to receptor sites to account for its prolonged pharmacological actions in the horse.

Another unusual aspect of the pharmacological action of reserpine is its very steep dose-response curve, which has a midpoint slope of at least 2.6 (Figure 5). Since the steepest midpoint slope of a dose-response curve for a 1:1 pseudo-irreversible drug receptor interaction is 1.15,³ this is an exceptionally steep dose-response curve. The most likely explanation for this anomaly appears to be a threshold phenomenon, i.e. occupation of a substantial fraction of binding sites before an effect on uptake is observed.³ Because the pharmacological effect then results from occupation of a relatively small proportion of the total number of receptor sites, the slope of the dose-response curve can be of the same order as that in Figure

5, independently of whether or not the drug-receptor interaction is reversible or irreversible.³

The dose of reserpine required to inhibit the uptake of serotonin by horse platelets was unexpectedly low. A dose of 0.5 mg/1000 lb reserpine produced essentially maximal inhibition of platelet serotonin uptake, but this dose gave rise to no clearcut signs of reserpinization. The reason for this discrepancy is not clear. Perhaps the simplest answer to this dilemma is that serotonin may not be involved in the tranquilizing actions of reserpine. Work reported by Roth and others suggests that tranquilization produced by reserpine may be more closely related to norepinephrine metabolism than to serotonin metabolism.¹ In any event, it is clear that platelet serotonin metabolism is unusually susceptible to reserpine and is a very sensitive indicator of reserpinization in horses.

As a practical matter, these results are not particularly helpful for the detection of reserpinization in horses. When this work was started it was hoped that inhibition of platelet serotonin uptake might prove to be a useful screening test for reserpinization in horses. Although the method is surprisingly sensitive, it remains an indirect method and is somewhat cumbersome. For this reason, the simple thin-layer screening test developed by Sams and Huffman at The Ohio State University provides an alternative screening test for reserpine in the horse.⁵ The principal importance of these results lies in the demonstrated rapid reversibility of reserpine's effect on horse platelets, especially at low doses, which may suggest that the concept of irreversible receptor binding of reserpine be reexamined.

⁴ Maylin, G.A.: Personal communication. New York State Racing and Wagering Board Drug Testing and Research Program.

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