fective when used in foals carrying various levels of related or unrelated maternal antibodies.

7. A comprehensive study of therapeutic agents routinely used in treatment of known foal pneumonia pathogens. Antibiotic evaluation should determine:
   a. The effectiveness of various antibiotics.
   b. The minimal inhibitory concentration needed.
   c. The length of time the antibiotic must be used to completely eradicate pathogen.
8. Evaluation of the effectiveness of various products, such as DMSO, Isoniazid and prostaglandins, that may be useful in the control of foal pneumonia. There is also a need to evaluate anthelmintics that may be effective against larval forms of helminths such as ascarids, strongyles and habronema.
9. Managerial research to improve the natural disease resistance of horses of all ages. This work should include:
   a. Establishment of the most disease-resistance-producing nutritional standards for all ages.
   b. Determination of the relative disease resistance of suckling foals and those fed other than their mothers' milk prior to weaning.
   c. Determination of the relative disease resistance of foals in relation to their weaning ages, and the influence of their post-weaning diets on disease resistance.
   d. Determination of the effects of various kinds of environmental and managerial stress upon foal health and resistance.
   e. Research into the selection of disease-resistant bloodlines.
   f. In the face of modern trends toward earlier weaning, studies are needed to more clearly evaluate nutritional levels afforded by mare's milk at various times of lactation.

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Pharmacology Review:

A Review of the Pharmacology of Reserpine in the Horse

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Reserpine is the principal alkaloid of a climbing shrub of the Apocynaceae family called *Rauwolfia serpentina*, which is indigenous to India and neighboring countries. It was described in ancient Hindu writings and medieval western medicine as the "insanity herb" and was widely used in traditional Indian medicine for treatment of hypertension, insomnia, and insanity.[12] These uses of the *Rauwolfia* plant went unnoticed by modern western medicine until 1931, when Sen and Bose described its use for treatment of psychoses and hypertension in Indian medical literature.[12] This report again attracted the attention of western investigators to *Rauwolfia*, and pure reserpine was isolated from the crude plant material in 1952. When reintroduced in this form, reserpine was the first of the modern major tranquilizers, and in the 1950s and early 1960s, was widely used in the treatment of psychoses and hypertension. It soon became apparent, however, that its use was associated with severe side effects. Thus, parkinsonism, severe depression, suicide, and seizures have been related to its use in humans, and reserpine is currently used only in occasional patients resistant to other forms of therapy.[13] In equines, however, reserpine is still widely used as a tranquilizer, because of its efficacy, difficulty of detection, and its long duration of action after a single dose.

Reserpine is a remarkably potent drug, and very small doses in the horse (< 5 mg) can produce considerable biochemical and behavioral effects. Reserpine is active in such low doses, and is so difficult to detect, that it was first thought to be a "hit-and-run" drug, *i.e.* a drug which when administered produced a biochemical change which persisted for days after the drug was eliminated from the body.[3] This concept is now known to be incorrect, and reserpine is known to be very tightly bound at specific sites in the body, and remains bound at these sites for at least as long as it produces its pharmacological effects, and possibly for much longer.[1]

Reserpine produces its actions in the brain by greatly reducing the brain content of certain neurohormones. Among the neurohormones whose

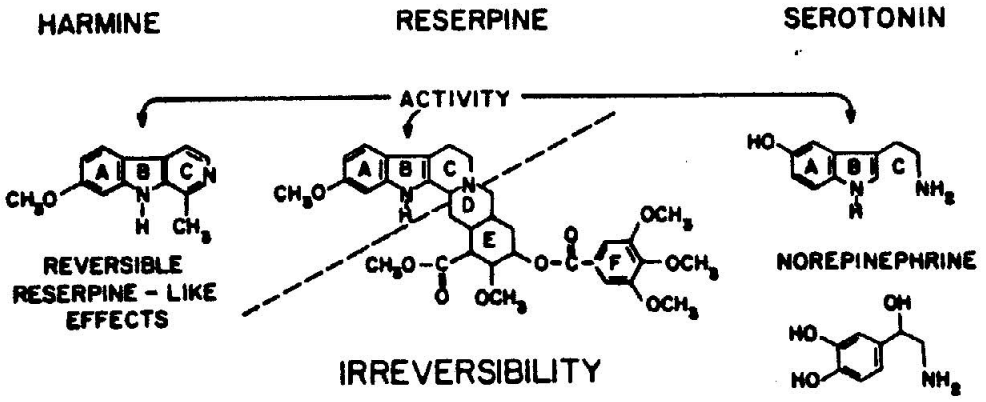

Figure 1. The structure of reserpine and related neurohormones. The A, B, C rings of the reserpine molecule are structurally related to the neurohormones norepinephrine and serotonin, whose storage in nerve terminals reserpine clocks. Harmine, which is structurally similar to the A, B, C rings of reserpine, produces the same pharmacological effects of reserpine, but its action is very short-lived. It therefore appears that the tight (irreversible) binding of reserpine and its prolonged action is associated with its D, E, F rings.

brain concentrations are reduced by reserpine are norepinephrine,[4] dopamine,[6] and serotonin,[10] and recent experimental work suggests that the behavioral effects due to reserpine are most closely linked to its depletion of brain norepinephrine,[5] although earlier work had suggested that serotonin was the neurohormone involved.[6] There appear to be good chemical reasons for this, because it turns out that one portion of the reserpine molecule (the A,B,C rings, Figure 1) is closely related structurally to norepinephrine and particularly to serotonin. Thus reserpine appears to displace norepinephrine and serotonin from their binding sites in nerve terminals, and in this way interferes with their function. Similar pharmacological and biochemical effects are also observed with harmine, a drug which is closely related structurally to serotonin (Figure 1), but whose effects do not last for more than a few hours.[13] It appears, therefore, that the D,E,F rings of the reserpine molecule are associated with the very tight binding of reserpine to its receptors, and it is this tight binding which gives reserpine its great effectiveness, potency, and its peculiarly long-lived actions.

While reserpine acts by depleting brain levels of catecholamines, it does so by depleting catecholamines from a storage granule which may account for some of the clinical characteristics of reserpine's actions. In catecholamine-containing neurons in the brain, the bulk of the catecholamines are found tightly complexed with ATP and Mg^{++} in granules (Figure 2).[13] It is these granular binding sites which are affected by reserpine. Reserpine binds tightly to these granules, and in so doing disrupts their structure and their ability to bind catecholamines. Because norepinephrine is an excitatory neurohormone in the brain, its depletion readily explains the calming or tranquilizing effect of reserpine in the horse. It has been calculated that each bound molecule of reserpine can cause the displacement of about 500 molecules of norepinephrine, which is then only very slowly replaced with newly synthesized norepinephrine. Other estimates

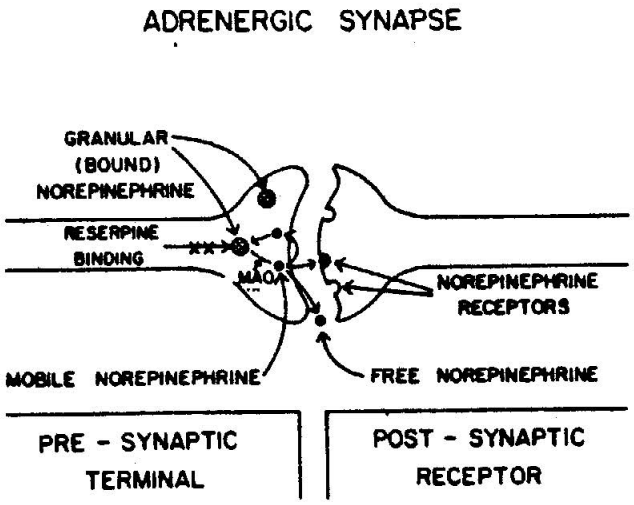

Figure 2. The site of action of reserpine on adrenergic synapse. Norepinephrine (or serotonin) is tightly bound in granules in the presynaptic terminal. Reserpine binds to these granules, disrupts them, and thus displaces the bulk of the neuronal norepinephrine. Normally, this released norepinephrine is metabolized by monoamine oxidase (M.A.O.), so it does not reach the postsynaptic receptor and result in central nervous system excitement. However, if monoamine oxidase has been blocked by a monoamine oxidased inhibitor, the released norepinephrine reaches the postsynaptic receptor causing excitement and the so-called "reserpine reversal."

suggest that only about 20 reserpine molecules are bound to each affected granule, further emphasizing the great potency of this drug.[1] The norepinephrine and catecholamines released by reserpine are usually hydrolyzed by monamine oxidase before they reach the postsynaptic receptors (Figure 2), and thus the release by reserpine of excitatory neurohormones is associated with minimal signs of central nervous system excitation in the horse.

This mechanism of action, however, leads to an unusual variation in the action of reserpine called "reserpine reversal." It turns out that if one administers a monamine oxidase inhibitor such as phenelzine or iproniazid to an animal prior to reserpine, all the caecholamines displaced from the granules are not metabolized and readily reach the postsynaptic membrane, where they cause central nervous system stimulation. Under these circumstances, reserpine can cause marked excitation instead of depression, giving rise to the name "reserpine reversal" for the effect.

Most of the experimental data on which this mechanism of action was developed is based on experiments on brain tissue and behavior in small laboratory animals.[13] However, it turns out that blood platelets also possess serotonergic granules and actively store serotonin[14] in much the same way as brain tissue. Because of this, in our studies on the actions of reserpine in the horse, we investigated the effects of reserpine on serotonin uptake in horse platelets as a biochemical correlate of the pharmacological effects of this drug in the horse.

When 10 mg of reserpine is administered intravenously (IV) to a horse, little change is seen for the first two hours. The first signs observed are usually sweating over the shoulder, back, stifle, and inguinal areas, at about three hours. Shortly thereafter the animal starts to pass considerably increased amounts of gas, and increased gastrointestinal motility and diarrhea commences. Slight ptosis (drooping of the eyelids) appears, increases with time, and remains one of the most enduring and sensitive indications of reserpinization. If the animal is male, penile extension starts at about five hours, is maximal (for this particular dose) at about 12 hours, and may be almost retracted by 24 hours.

At about four hours after dosing, the animal has a glazed appearance and by eight hours may be standing relatively immobile and apparently depressed. If the animal is particularly sensitive to reserpine (and there are suggestions that males are more sensitive to reserpine than females) it may show signs of acute colic and go down for a period. More commonly, however, the animal simply appears depressed and may appear so for up to 48 hours. By 60 hours postdosing, however, animals dosed with 10 mg of reserpine will appear clinically normal.

It is characteristic of the pharmacology of reserpine that animals sedated with this drug are easily aroused,[13] and Figure 3 shows a rather clear-cut demonstration of this effect in the horse. As pointed out previously,[17] the narcotic fentanyl produces a very clear-cut behavioral stimulation pattern in the horse. In Figure 3 a horse was challenged with fentanyl before and after treatment with reserpine. Despite the marked depression of spontaneous motor activity by reserpine, challenge with fentanyl after reserpine produced almost exactly the same motor response as before reserpine. Thus, though reserpine served to markedly reduce the spontaneous activity of the horse, it did not in any way affect the response of this animal to fentanyl.

In our hands, lower doses of reserpine produced correspondingly less marked pharmacological effects.

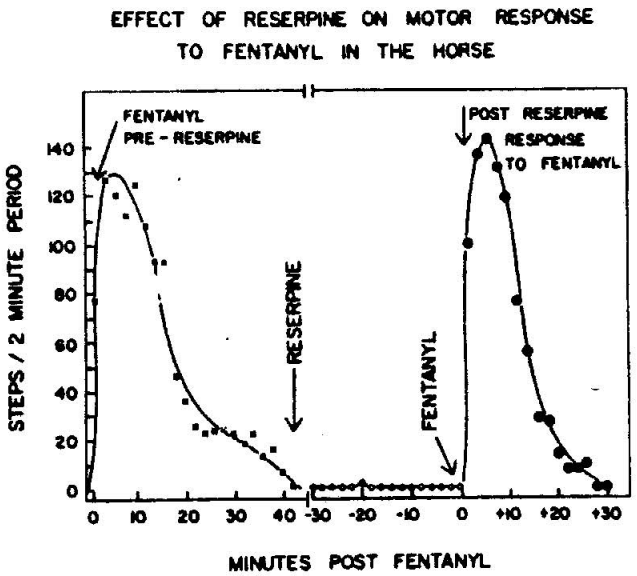

Figure 3. The effects of reserpine on spontaneous and fentanyl-induced motor activity in the horse. The left-hand portion of this figure (crosses, X - X) shows the marked increase in spontaneous motor activity induced in this horse by rapid IV injection of 8 mg Fentanyl Citrate. This animal was then treated with 10 mg reserpine IV, and the open circles (O - O) show the lack of spontaneous activity in this horse three hours after reserpine, the animal making one step in a 30-minute period. However, when again challenged with 8 mg of fentanyl, the animal responded as previously with no apparent diminution in response. Activity was measured by counting steps/2-minute period as described previously.[16]

A dose of 5 mg IV produced droopy eyelids at three hours with passage of gas and some diarrhea at about eight hours. A dose of 0.5 mg to a horse produced little observable change beyond the passage of some very loose stool at about 10 hours. At these low doses (0.5 to 1 mg) the animals were undistinguishable from untreated horses within 24 hours. These dose-response relationships agree well with some very early dose-response relationships reported in the horse by Earl.[8]

In experiments in small laboratory animals, reserpine shows similar calming effects, and while the animals may sleep, they are easily aroused and are responsive to external stimuli. Also, while spontaneous activity is decreased, motor coordination is not affected.[13] Our observations in the horse, as presented in Figure 3, correlate well with these reports.

In the peripheral nervous system most of the actions of reserpine are consistent with catecholamine depletion, and may be explained by a decrease in sympathetic tone. In the circulatory system reserpine produces hypotension and bradycardia, and these are the principal pharmacological effects of reserpine currently being utilized in human medicine.[13] In the gastrointestinal tract, reserpine causes increased frequency of defecation and diarrhea, due to decreased symphathetic and increased parasympathetic tone, effects readily observable in horses. Currently, reports in the literature of deaths due to reserpine are rare,[13] but the horse appears to be particularly susceptible.[4] Though Earl[8] reports statements that 5 mg can produce violent colic in a horse, we have routinely used 10 mg in our studies with no problems, so the lethal dose for the horse must be substantially above this. The recommended antidote to reserpine is methamphetamine,[3] which makes good pharmacological sense in view of the fact that the primary pharmacological action of reserpine is to deplete norepinephrine.

Since we could not sacrifice our horses for neurohormone assays, we elected to follow the biochemical effects of reserpine in these horses by studying the effects of reserpine on platelet serotonin.[7,14,15] It turns out that blood platelets have a serotonin uptake and storage mechanism similar to those found in serotonergic neurons in the brain, and this uptake mechanism is also sensitive to reserpine.[14] Analyzing the ability of horse platelets to take up radio-labeled serotonin before and after reserpine treatment, White and Tobin[19] found that this uptake was up to 75% inhibited six hours after a dose of 10 mg of reserpine, remained depressed for at least three days, and returned to control by seven days (Figure 4).

The most likely explanation for these effects is that reserpine is bound to the serotonergic granules in the platelets, which shows up as the inhibition of platelet serotonin uptake. This uptake was maximally depressed at six hours after an IV dose, and thereafter recovered slowly.[20] Because platelets in the horse live only about 14 days, in contrast with the lifetime existence of nerve cells in a horse, this is likely to be the shortest period for which the central effects of reserpine on brain norepinephrine and serotonin metabolism may be observed in the horse. It is also likely to be the shortest period for which reserpine persists in the body of the horse. It further turns out that horse platelets are surprisingly sensitive to reserpine, and doses of only 1 mg of reserpine or less produce an approximately similar effect.[20]

These actions of reserpine in the horse are quite consistent with its basic mechanism of action. Because

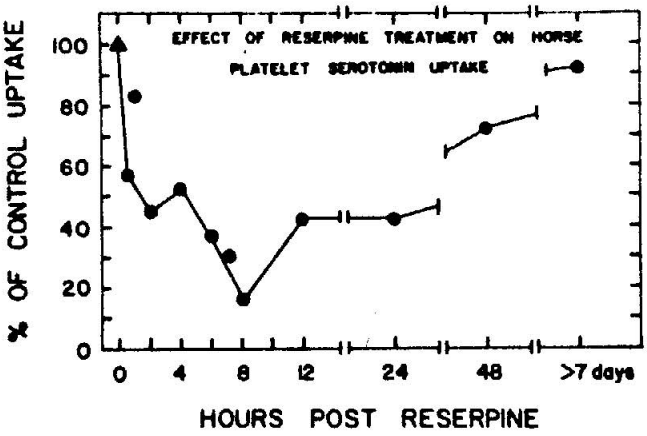

Figure 4. The effect of reserpine treatment on [^{3}H] serotonin uptake by equine platelets. The initial rates of [^{3}H] serotonin uptake were measured as described by White and Tobin.[19] The zero time value (solid triangle ▲) represents the initial rate of [^{3}H] serotonin uptake in platelets drawn from this horse prior to treatment with reserpine. Platelet uptake of serotonin is measured as mm [^{3}H] serotonin/10^{11} platelets/min, and the control value is expressed as 100%. The solid circles (● - ●) show the depression in the initial rate of serotonin uptake by platelets drawn from this horse at the indicated times after treatment with 5 mg of reserpine by rapid IV injection. These results are typical of experiments on a number of horses, and broadly similar results are seen after 10 mg and 1 mg of reserpine.

granular norepinephrine in the synapse is not directly available for neurotransmission, a lot of reserpine must be bound, and norepinephrine depleted from the granule, before pharmacological effects are seen. Thus, one might expect platelet serotonin uptake to be at least as sensitive, if not more sensitive, an indicator of reserpinization than the animal's behavior. Since the mobile pool of norepinephrine in the nerve termi-

nals is not directly affected by reserpine, animals respond well to drugs or specific stimuli in a nearly normal manner, as was seen with fentanyl.

Although the plasma half-life of reserpine in the horse has not yet been determined, it is likely to be very long. The first reason for this is that reserpine is a highly lipid-soluble drug[13] which distributes very widely in the body. A lipid-soluble drug can get into every nook and cranny in the body, and it simply takes the body's metabolizing systems a long time to find and eliminate it. Secondly, reserpine is very tightly bound at its specific binding sites on the catecholamine storage granules.[1] It is not at this time clear whether or not this tightly bound reserpine is ever free again and is excreted, or even if this pool of reserpine is big enough to significantly affect the excretion pattern,[13] but its effect can only favor a longer half-life for this drug in the horse. Studying the plasma half-life of reserpine in humans, Maas and co-workers[9] reported a 271-hour (11-day!) half-life for reserpine in humans, and detected reserpine in plasma, urine, and feces 12 days after administration of 0.25 mg to humans. As pointed out earlier, there is no reason to suspect that reserpine will be any less persistent in the horse, and it may turn out that after a course of therapy reserpine will be detectable in the body of the horse for very long periods indeed.

Because reserpine is so persistent in the horse and has such a prolonged pharmacological action, it is possible to very accurately control the amount of reserpine in a horse, and the pharmacological effects attained. Thus daily administration of low-milligram doses of reserpine should allow the gradual accumulation of reserpine in the horse, and a gradual increase in the pharmacological effect. At any point during this slowly increasing effect, the effect may be maintained by simply reducing the dose rate by about 50% or more to a maintenance dose. If dosing is abruptly stopped, the drug effect will decay at the fastest possible rate, the same as that observed after an IV dose, though the pharmacological effects may take several days to dissipate and blood levels of the drug even longer.

Until very recently, no analytical test for the presence of reserpine in the horse was available, and because of this its use in competition horses could not be controlled. Recently, however, Sams and Huffman at Ohio State University have reported on a thin-layer screening method for detection of small doses of reserpine in the horse.[11] This is basically a micro-thin layer modification of a method described by Tripp and co-workers,[18] and it is reported to detect low levels of reserpine in equine plasma (but not urine) for several days after a dose of reserpine. Although this test represents a major advance in analytical testing for reserpine, it must be kept in mind that thin-layer chromatography does not chemically identify a substance. This lack of specificity can lead to the occurrence of false positives and thus to problems with its use as an unequivocal forensic test for reserpine.[a] Thin-layer chromatographic tests are not specific tests,[16] but merely tests which can eliminate compounds, *i.e.* tests which can prove the absence of a drug but not its presence. According to Stein *et al.*,[16] "if TLC is to be used for identification purposes, all possible alternative compounds, including derivatives and isomers, must be chromatographed and their Rf values found to be different from the unknown suspected material before a positive identification can be made."

The requirement for positive chemical identification is particularly important in the case of reserpine, since this drug is a plant product. Plants growing in the southeastern United States which have been reported to contain reserpine include the periwinkles, members of the genus *Vinca*, such as Vinca herbacea, and Vinca rosea. In addition, there are about ten or a dozen species of the Apocynaceae growing in the southeastern United States, but knowledge concerning the reserpine content of these plants is limited.[b] In view of the possibility of "positives" in equine plasma from reserpine or related akaloids of botanical origin, particular care should be taken by analysts when testing for reserpine in equine plasma.

[a] Personal communications from Dr. George Maylin and Dr. Jack Henion, NYS Racing and Wagering Board, Drug Testing and Research Program, Cornell University, Ithaca, NY, and Mr. John McDonald, Chief Chemist, Illinois Racing Commission, 160 N. LaSalle Street, Chicago, IL.

[b] Personal communication from Professor Trevor Robinson, Department of Biochemistry, University of Massachusetts.

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After one hour, cultures of bacteria were again applied and incubated. The mean colony count showed that Nolvasan Surgical Scrub maintained high residual activity—higher than the other two scrubs against *E. coli*, but dramatically higher than povidone-iodine against *Staph. aureus*. Previous studies have shown that Nolvasan retains high antimicrobial activity even in the presence of blood and other organic matter, to a much greater extent than most other disinfecting agents.

(continued on page 444)