

Pharmacokinetics and Behavioral Effects of Methylphenidate in Thoroughbred Horses

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SUMMARY

In horses given (rapid iv) methylphenidate (Ritalin, α -phenyl-2-piperidinacetic acid methyl ester; 0.70 mg/kg), plasma concentrations of the drug decreased rapidly at first, with an apparent α half-life of about 19 minutes, and then more slowly, with an apparent β half-life of about 2.4 hours. These data were well fitted by a 2-compartment open model. In blood, about 40% of the methylphenidate present was in the plasma fraction, and of this, about 80% was plasma-protein bound. If given by subcutaneous or IM injection, plasma concentrations of methylphenidate peaked in about 1 hour and were no longer detectable (cleared) from plasma by 6 hours. Urinary clearance time, however, was longer, and between 12 and 24 hours should be allowed for a dose of methylphenidate to "clear" from urine. Using a variable-interval responding apparatus, methylphenidate was shown to stimulate the responding rate of horses up to 6 times above base line, making it the most potent central stimulant tested in this responding apparatus to date. Peak central stimulation at 30 minutes after IV dosing was seen in horses given 0.4 to 1.0 mg of methylphenidate/kg.

Methylphenidate (α -phenyl-2-piperidinacetic acid methyl ester, Ritalin), is a mild CNS stimulant which is considered, in persons, to have more prominent effects on mental than on motor activity. Although methylphenidate produces pharmacologic effects broadly similar to those of amphetamine, there are important differences both in the basic mechanisms of action of these drugs and in their clinical use.¹ In human medicine, methylphenidate is widely used for control of hyperkinetic children.¹

In veterinary medicine, methylphenidate has reportedly been used in racing and in show horses to improve performance or demeanor of these animals.² Use of methylphenidate

in this way is prohibited by racing authorities and show-horse associations. As part of a study of the performance and behavioral effects of stimulant drugs in horses, the pharmacokinetics and related behavioral effects of methylphenidate are described.

Materials and Methods

Mature Thoroughbred horses, weighing between 450 and 550 kg, were used. For the experimental period, these animals were housed in individual box stalls and fed hay and water ad libitum. All drug administrations, behavioral observations, and sample collections were performed as described.² The IV injections and blood sample collections were done by jugular venipuncture; IM injections were made deep in the muscles of the lateral cervical region; subcutaneous (SC) injections were made between the thoracic limbs over the superficial pectoral muscles. Urine samples were collected by catheterization of the urinary bladder or occasionally during spontaneous voiding of urine. Methylphenidate for injection was commercially lyophilized Ritalin hydrochloride,^b as was authentic methylphenidate HCl (lot M-6379) used in the preparation of standards and for the in vivo experiments.

Methylphenidate concentrations in biological fluids were determined by the method of Blake et al.³ To 4 ml of the biological fluid or appropriate aqueous standards, 1 ml of saturated sodium tetraborate and 2 ml of cyclohexane were added. The mixture was then agitated for 5 minutes and centrifuged. Occasionally, the agitation produced a partial emulsion which was resolved by centrifuging at 20,000 g for 20 minutes. After centrifugation, the cyclohexane layer was transferred to another tube, and 50 μ l of heptafluorobuturic acid was added; this mixture was allowed to react at room temperature for 3 minutes. At the end of this period, 5 ml of 0.5 M NaOH was added to the mixture, which then was again rotoracked for 10 minutes. After centrifugation, the cyclohexane layer was transferred to a clean tube and an aliquot taken for gas chromatography. Gas chromatography was performed on a Perkin-Elmer 3920B chromatograph equipped with an electron capture detector. Separation was done on a 3% OV-101 column^c at an injection temperature of 250 C, a column temperature of 220 C, and a detection temperature of about 300 C. Under these conditions, the retention time for derivatized methylphenidate on the column averaged about 3 minutes. Typical standard curves for the recovery of methylphenidate from water, plasma, and urine are shown in Figure 1. Unless otherwise stated, all experimental points are the means \pm SEM of at least 4 separate experimental determinations.

Behavioral Experiments—The central stimulant actions of methylphenidate were quantitated, using an operant behavioral method as described by Shults et al.² In this method, horses are trained to break an electric light beam for rewards (oats). The trained horses are rewarded on a variable-interval schedule and

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^a Blake JW, Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington: Personal communication, 1978.

^b Ciba-Geigy Ltd, Summit, NJ.

^c Applied Science Laboratories Inc, State College, Pa.

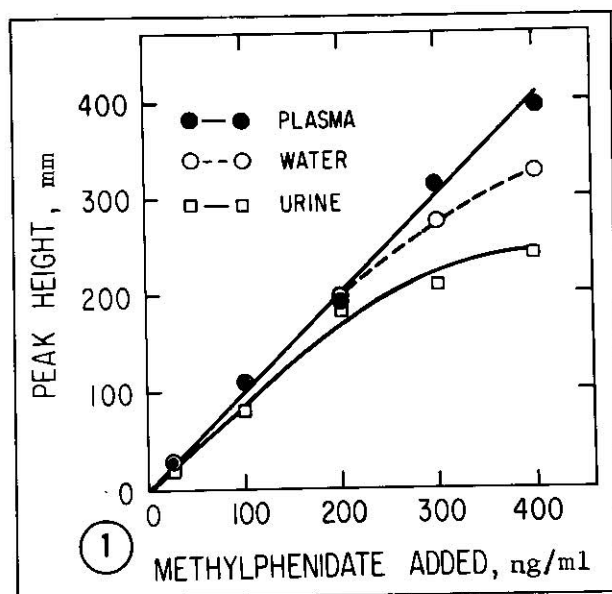


Fig 1—Recovery of methylphenidate from water, plasma, and urine. The indicated concentrations of methylphenidate were added to aliquots of distilled water, plasma, or urine. Recovery of methylphenidate from plasma is indicated by the solid circles (●—●), from water by the open circles (○—○), and from urine by the open squares (□—□).

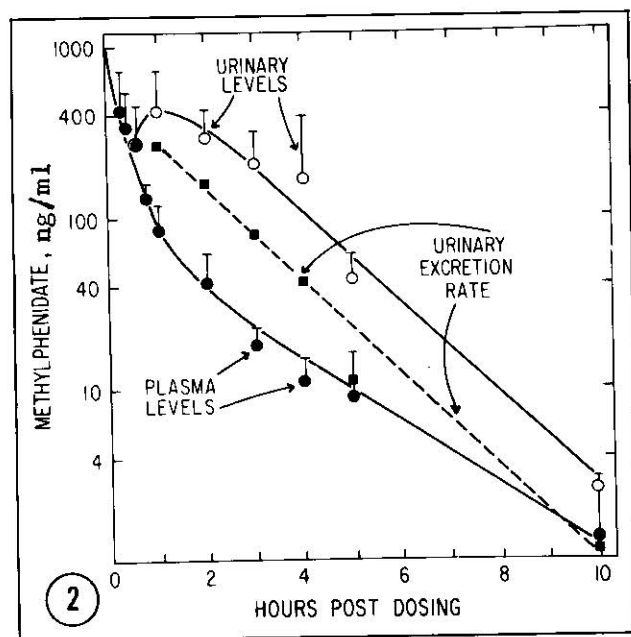


Fig 2—Plasma and urine concentrations of methylphenidate after rapid iv injection of 0.7 mg/kg. Methylphenidate (0.7 mg/kg) was administered by rapid iv injection to horses, and blood and urine samples were taken at the indicated times. The solid circles (●—●) show plasma concentrations of methylphenidate at the indicated times after injection, and the open circles (○—○) show urinary concentrations. The solid squares (■—■) show the urinary excretion rate of the drug in $\mu\text{g}/\text{ml}/\text{hour}$ at each time. All experimental points are means \pm SEM of experiments on 5 horses.

exhibit a consistent and stable responding rate which serves as a control responding rate (behavior) for drug studies. (The equipment used in this method is referred to as the VI apparatus.^d)

Horses were tested on the VI apparatus each morning for 30 minutes. Methylphenidate or normal saline solution was administered 20 minutes before testing, and saline solution injections were repeated on at least 3 days before each drug administration. Control responding rates did not fluctuate more than $\pm 10\%$ throughout the experiments.

^d Coulbourn Instruments, Lehigh Valley, Pa.

TABLE 1—Mean Kinetic Values Calculated from Data of Figure 2

Symbol	Value
A	711 ng/ml
B	38.2 ng/ml
α	2.22 hr^{-1}
$t_{1/2\alpha}$	18.75 min
β	0.295 hr^{-1}
$t_{1/2\beta}$	2.35 hrs
K	1.593
k_{21}	0.331
k_{12}	0.725

Data were fitted to 2-compartment open model (SAAM-23) for individual horses and then averaged.

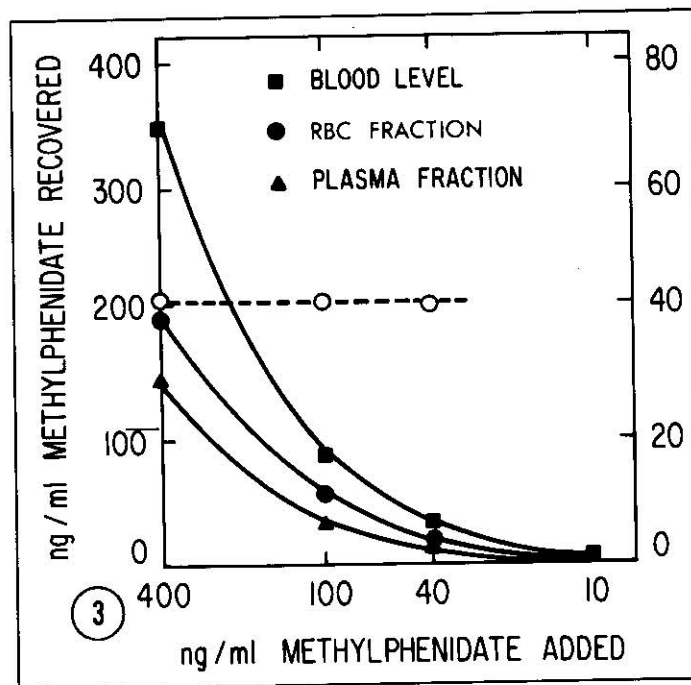


Fig 3—Blood-plasma partitioning of methylphenidate. The concentrations of methylphenidate indicated on the horizontal axis were added to 10 ml of freshly drawn equine blood and incubated at 37 C for 10 minutes. The samples were then centrifuged at $5,000 \times g$ for 10 minutes, and the RBC fractions were separated. The solid circles (●—●) show the concentrations of methylphenidate in the RBC fraction, and the triangles (▲—▲) show the concentrations in the plasma fraction. The squares (■—■) show the mean concentrations of methylphenidate recovered, and the open circles (○—○) show the percentage of the drug present in the plasma fraction at each drug concentration. Each point is the mean of 3 determinations.

Results

Figure 1 shows typical standard curves for the recovery of methylphenidate from water, equine plasma, and equine urine. Essentially similar recoveries were obtained from plasma and aqueous samples "spiked" with known amounts of drug added, but recovery from urine was usually less, particularly with the higher drug concentrations. Because of this reduced recovery and the inherent day-to-day variability in chromatographic column performance and detector response, complete standard curves from "spiked" plasma and urine were run in association with each experiment reported.

Figure 2 shows plasma and urine concentrations in horses given methylphenidate by rapid iv injection at a dosage of 0.7 mg/kg of body weight. The decrease in plasma concentrations of methylphenidate was biphasic, showing an initial rapid decrease over the 1st hour and then a slower rate of decrease. Fitting the plasma concentrations to a 2-compartment open model (Table 1), using a SAAM-23

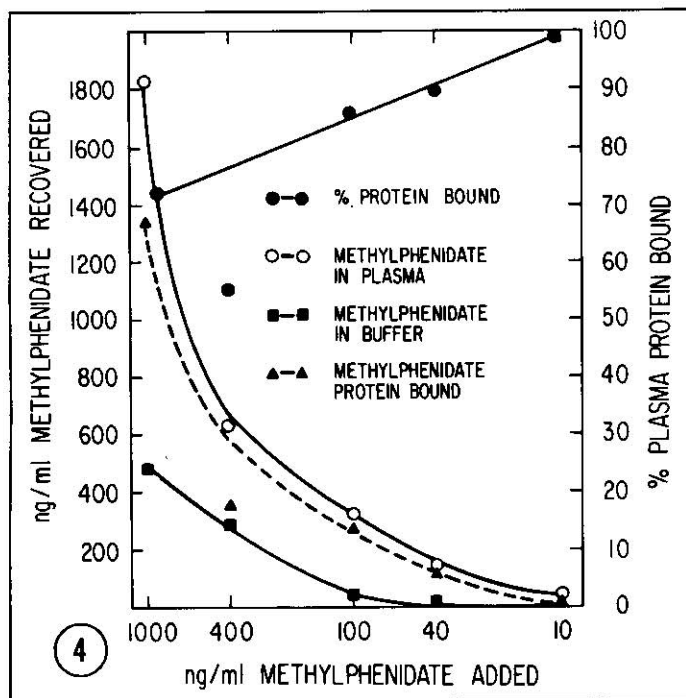


Fig 4—Binding of methylphenidate to equine plasma protein. The indicated concentrations of methylphenidate were added to 45 ml of 50 mM phosphate buffer (pH 7.4), to which 5 ml of freshly drawn equine plasma in a spectrophor dialysis bag was added. The system was incubated at 37 C for 4 hours with vigorous shaking. Preliminary experiments showed that 4 hours was adequate for equilibration of methylphenidate across the dialysis membrane. At the end of the incubation period, 4-ml aliquots of the plasma and buffer were extracted and their methylphenidate content was assayed, as described in Materials and Methods. The solid squares (■—■) show the concentration of methylphenidate recovered from the aqueous phase, and the open circles (○—○), the concentration of methylphenidate in the plasma phase. The difference between these values, the plasma protein-bound methylphenidate, is indicated by the triangles (▲—▲). The solid circles (●—●) show the percentage of methylphenidate protein bound at each concentration added to the system. All data points are the means of 4 individual experiments.

program,⁶ yielded a good fit of the data with an apparent α -phase half-life of about 19 minutes and a β - or metabolic phase half-life of about 2.4 hours. Urinary concentrations of methylphenidate peaked (about 400 ng/ml) at 1 hour and thereafter decreased approximately in parallel with the plasma concentrations.

To determine the true volume of distribution of methylphenidate in the horse to assist in its forensic detection in blood samples, studies of its blood-plasma distribution and plasma-protein binding were made. Figure 3 shows the distributions of methylphenidate in plasma and in RBC over a range of blood concentrations of added drug. The experiment indicates that about 60% of the methylphenidate present in blood *in vitro* partitions into the RBC.

Figure 4 shows the proportions of free and bound methylphenidate present in plasma. At the high plasma concentration of 1,000 ng of methylphenidate/ml, about 70% was plasma-protein bound, and at pharmacologic concentrations (400 ng/ml or less), methylphenidate was more than 80% plasma-protein bound.

Because methylphenidate is presumably given by sc or im injection to racing or show horses, we studied its dispo-

⁶ Berman M, Weiss MF: *Users Manual for SAAM*. National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md, 1968.

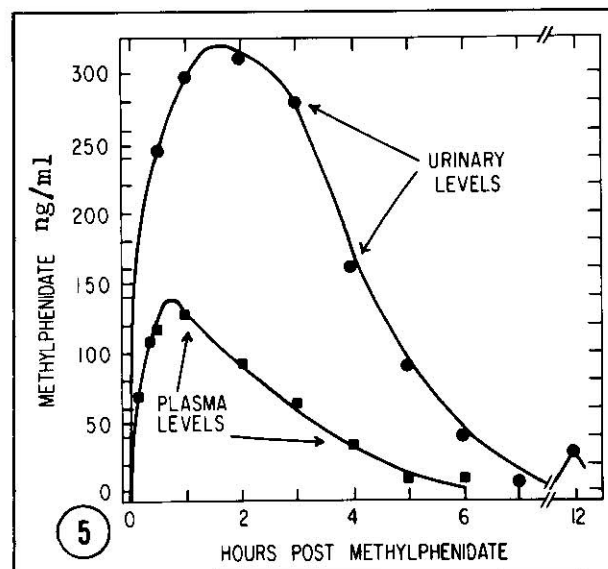


Fig 5—Plasma and urine concentrations of methylphenidate after sc injection of 0.35 mg/kg. Methylphenidate HCl (0.35 mg/kg) was administered by sc injection to 4 horses. The solid squares (■—■) show plasma concentration of the drug, and the solid circles (●—●) show urinary concentrations. All data points are the means of experiments on 4 horses.

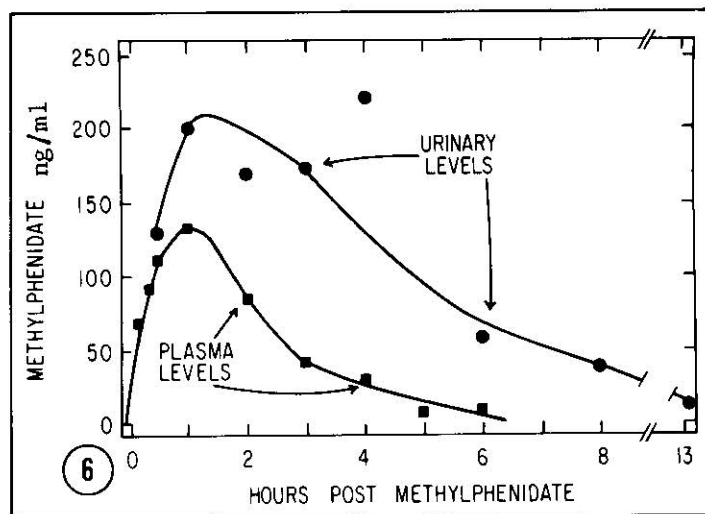


Fig 6—Plasma and urine concentrations of methylphenidate after im injection. Methylphenidate HCl (0.35 mg/kg) was administered by im injection to 4 horses. The solid squares (■—■) show plasma concentrations of the drug, and the solid circles (●—●) show urinary concentrations. All data points are the means of experiments on 4 horses.

sition after injection by these routes. After sc injection of 0.35 mg of methylphenidate/kg, plasma concentrations of the drug increased slowly, peaked at about 1 hour, and then decreased with a half-life of about 1.5 hours (Fig 5). In the urine, drug concentrations peaked after 2 hours, at about 3 times the plasma concentrations, and then decreased rapidly with an apparent half-life of about 1.0 hour. For horses given the same dose of methylphenidate by im injection, the time course and the plasma and urinary concentration of the drug were broadly similar to those obtained in horses given the drug by sc injection (Fig 6).

After im injection of methylphenidate, marked transient changes in urine volume and concentration were noted. These effects appeared at about 30 minutes and persisted for about 2.5 hours. Coincident with these urinary changes,

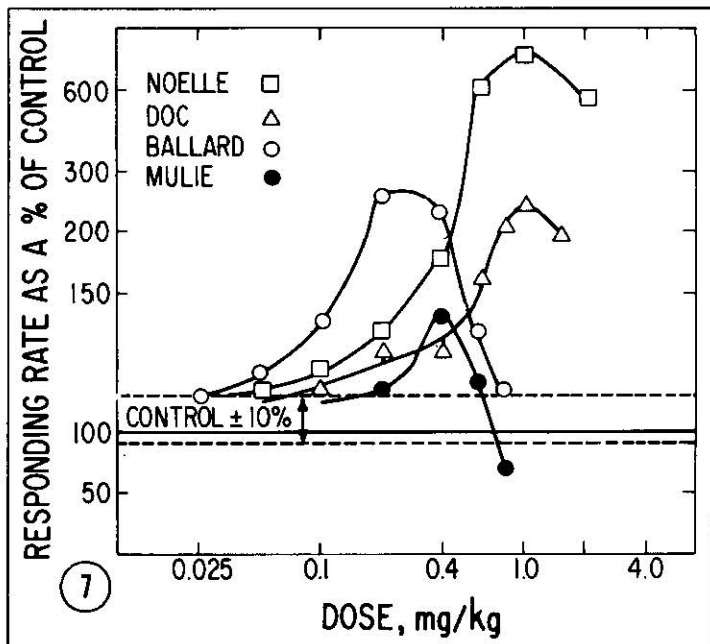


Fig 7—Effect of methylphenidate on responding rate in a VI-60 schedule. Four horses were dosed with the indicated amounts of methylphenidate iv and introduced to the responding apparatus 20 minutes later. The symbols show the percentage of change in rate for each 30-minute session after the indicated dose of methylphenidate was given to each of 4 horses. Each experiment was performed between 10 and 12 AM on consecutive days, and horses were treated with saline solution and allowed to return to their control responding rates between tests.

a large electron-capturing peak with a retention time of about 5 minutes was noted. This peak became larger up to about 1 hour and then decreased with a half-life of about 90 minutes. The substance giving rise to this peak was not further identified.

Figure 7 shows the effects of increasing doses of methylphenidate on the responding rate of 4 horses on a VI-60 schedule. Doses of methylphenidate of about 0.1 mg/kg were approximately threshold doses for increases in the responding rate. For 2 horses given a dose of 0.4 mg/kg, the responding rate peaked at between 150% and 250% of control before decreasing. For 2 other horses, the responding rate reached peak with doses of about 1 mg/kg, for 1 horse at 250% of control and for 1 horse at about 650% of control.

Discussion

After rapid iv injection, plasma concentrations of methylphenidate decreased rapidly, with an apparent half-life of about 19 minutes initially, and then more slowly with an apparent β half-life of about 2.4 hours. In blood, about 40% of the methylphenidate was in the plasma fraction, and about 90% of the methylphenidate in the plasma fraction was protein bound. After sc and im administrations of the drug, plasma concentrations of methylphenidate peaked within 1 hour and then decreased, while urinary concentrations peaked at 2 hours at between 2 and 3 times plasma values. Thereafter, urinary concentrations decreased at about the same rate as did the plasma concentrations. Overt behavioral signs of central stimulation were minimal at all plasma concentrations of the drug, but no rigorous quantitation of behavioral signs was attempted. Studies with a VI-60 schedule in a variable-interval responding apparatus indicated that doses of methylphenidate greater than 0.1

mg/kg were associated with increased responding rates in the most responsive horses, whereas maximal stimulation was associated with a dose of 1.0 mg/kg. Each horse tested, however, had its own particular response pattern to methylphenidate.

These observations on the pharmacokinetics of methylphenidate in horses are in good agreement with those reported by Ray et al.⁴ These workers measured plasma and urinary concentrations of methylphenidate after im administration of 150 mg of methylphenidate HCl to horses of unknown weight. Assuming an average weight of about 450 kg for those animals, Ray et al may have given the drug at a dose rate of 0.33 mg/kg. As in the present study, plasma methylphenidate values were at peak in an hour and then decreased with an apparent half-life of about 1.3 hours. Mean peak blood values (120 ng of methylphenidate/ml) reported by Ray et al are somewhat less than the 200 to 300 ng/ml observed in our experiments.

Overt behavioral signs of CNS stimulation were not seen in the present studies, and similarly, Ray et al⁴ did not report on any clinical signs of excitation in animals used in their studies. The only published report on the clinical changes attributable to methylphenidate administration in the horse is that of Gabriel et al.⁵ These investigators administered doses of up to 600 mg of methylphenidate im. Methylphenidate given in doses of 50 mg produced doubling of the respiratory rate of the horses within 15 minutes, but the rate of increase decreased over the next hour. In horses given 400 mg, the respiratory rate peaked at 60 breaths/minute and had not returned to base line by the 2nd hour. In horses given the dose of 600 mg, the respiratory rate was too rapid to count for the first 45 minutes. Changes in pulse rate were not seen until the 400-mg dose was administered and the pulse rate was more than doubled at the 600-mg dose. However, these authors saw no significant changes in the ECG or the hemogram at any of these doses.

Gabriel et al⁵ reported that signs of CNS stimulation in the horse were not seen until about 500 mg of drug was administered, at which dose the horses tended to become restless. However, these authors made no attempt to quantitate these effects. In 1 horse, they measured the effects of 100 and 200 mg of methylphenidate on blood pressure and found that both doses increased blood pressure by about 35%, with the effect peaking between 30 and 45 minutes after administration of the dose. Gabriel et al concluded that methylphenidate was capable of producing CNS stimulation in the horse at dose levels as low as 50 mg, and considered that the behavior of the animal was so affected by the larger doses that it would be relatively easy to pick out illegally medicated horses. However, it appears that relatively large doses in the order of 1 mg/kg are required for such effects, and Gabriel et al did not discuss how a methylphenidate-medicated horse could be identified differentially from a spontaneously nervous horse.

In our initial kinetic studies, methylphenidate in doses of up to 0.7 mg/kg did not produce readily observable signs of CNS stimulation due to Ritalin. In particular, clear-cut locomotor activity, so readily apparent after narcotic analgesics and dopaminergic agonists, was not observable. However, when Ritalin-treated horses were tested in the responding apparatus, clear-cut signs of behavioral stimulation were seen in all horses, with clear-cut individual differences in sensitivity between horses. Thus, the most sensitive horse showed a 40% increase in responding rate at

0.1 mg/kg and his response peaked between 0.2 and 0.4 mg/kg and then decreased. Another horse responded poorly to methylphenidate and yielded a peak response of 30% above control at 0.4 mg/kg, after which the response was depressed. Two other horses showed peak responding at a dose of 1.0 mg/kg, and 1 of these horses yielded by far the largest stimulation of responding rate seen with any drug. This very large increase in responding rate is consistent with what is known about methylphenidate as a drug which increases the ability to concentrate on a desired behavior, as well as being a central stimulant.⁶

The only report on effects of methylphenidate on performance in the horse is that by Sanford^f who reported that methylphenidate increased speed of horses given doses which had little effect on coordination. Seemingly, methylphenidate produced this effect in short-gallop screening tests on stimulant drugs, but details of drug dose or magnitude of the effect were not given.

As a practical matter, these results indicated that methylphenidate is rapidly "cleared" from plasma of horses given the drug by IV, SC, or IM routes. "Clearance" from urine occurs more slowly, with at least 12 hours being required for clearance, and more likely as long as 24 hours. The overt signs of CNS stimulation in horses on methylphenidate are small. In our experience, they do not compare with the

^fSanford J: Drugs and their effects on performance in the horse. Horserace Betting Levy Board Conf Res Workers, April 1973, pp 40-41.

locomotor stimulation induced by apomorphine or the narcotic analgesics or with the restlessness in horses given large doses of cocaine. When tested in the responding apparatus, however, clear-cut signs of CNS stimulation were seen. With high-peak responding rates in horses given methylphenidate by SC or IM injection, peak plasma concentrations occur about 1 hour later, which indicates that peak behavioral effects should be found at about this time after dosing by this route.

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