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THE PHARMACOLOGY OF NARCOTIC ANALGESICS IN THE HORSE. II. STUDIES ON THE DETECTION, PHARMACOKINETICS, URINARY CLEARANCE TIMES AND BEHAVIOURAL EFFECTS OF PENTAZOCINE AND FENTANYL IN THE HORSE

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Summary

After intravenous injection, pentazocine (Talwin) (1 mg/kg) distributed relatively slowly in the horse, with an α phase half-time of minutes, and a β, or metabolic terminal half-life of about 138 minutes. After intramuscular injection of 0.66 mg/kg, peak plasma levels occurred at about 30 minutes and plasma levels thereafter declined in a multi-exponential fashion with a very slow terminal half-life.

About 30 *per cent* of a dose of pentazocine administered to horses was eliminated in urine as a glucuronide metabolite. When analyzed for this metabolite, pentazocine was detectable up to five days after administration. Horsemen should be aware of "clearance time" for pentazocine to avoid racing within six days of pentazocine administration.

The opiate receptor was demonstrated in equine brain tissue with highest activity observed in the cerebrum and thalamus. Using the opiate receptor in a radio-receptor assay type experiment for fentanyl detection, equine urine was observed to contain materials which displaced (^{3}H) morphine from the opiate receptor which interfered with this assay.

The locomotor response to opiates was quantitated by counting footsteps of horses after dosing with fentanyl and pentazocine. After intravenous administration of fentanyl a clear-cut dose response to this drug at 20 μg/kg was observed. Pentazocine was much less effective than fentanyl in producing this motor response.

Introduction

The narcotic analgesics show a spectrum of pharmacological activities in the horse including analgesia, respiratory depression and a reputation for producing marked central nervous stimulation in the horse (Tobin, 1978). The pharmacokinetics, behavioural effects, analgesic actions and performance effect of this group of drugs in the horse are currently being investigated in our research programme. In this report we present a method for quantifying the behavioural stimulating effects of this group of drugs.

Pentazocine (Talwin^R) is a benzomorphan derivative which has strong analgesic activity and little dependence potential in man. It is

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Some of the data presented in this paper represent part of a dissertation by J. Richard Miller, submitted in partial fulfillment of the requirements for the degree Master of Science in Toxicology in the Graduate Toxicology Program, University of Kentucky, 1978.

reported to possess between one-fourth and one-sixth of the narcotic potency of morphine and to have a low incidence of side effects. It's pharmacokinetics have been studied in some detail in man and Davis and Sturm (1970) have reported on its plasma levels after intramuscular injection in various domestic animals, including the pony.

Fentanyl (Sublimaze^{R}) is a potent synthetic narcotic analgesic with a rapid onset and short duration of action. It has a profile of pharmacological activity similar to that of morphine, except that it does not cause emesis and histamine release. It is considered to be about 150 times more potent than morphine. After intravenous injection in man peak analgesia appears after 3-5 minutes and lasts about 60 minutes (Remington's Pharm. Sci., 1975). Unlike pentazocine, fentanyl is a Schedule II controlled substance.

Recently, the work of Snyder (Pert and Snyder, 1973) and others has resulted in identification of the narcotic receptor and its localization in nervous tissue of most vertebrates. Because this receptor material selectively binds narcotic drugs with high affinity it appeared to us that it might be possible to use this receptor material in a "radio-receptor" type assay for the presence of narcotic drugs in horse urine. This communication reports some studies on this possibility. Preliminary communications have been reported (Tobin and Miller, 1978).

Materials and Methods

(a) *Animals* — Care and maintenance of horses, administration of drugs and collection of blood and urine samples were as previously described (Tobin *et al*, 1977). Unless otherwise noted, all experimental points are the means±standard errors of the means of experiments on at least four different horses.

To determine spontaneous motor activity in the horse after administration of either pentazocine of fentanyl, horses were confined in a partially sound-proof box stall and observed through one-way glass. After being allowed a period of about 48 hours to become accustomed to the box the horses were dosed with the appropriate drugs or a saline control injection, usually intravenously. Then each time the animal lifted its left front foot from the ground a single count was recorded. To aid the observer in observing movements of the left foot it was wrapped from the knee down in white tape. Data points were recorded by the observer using a hand held counter and noted every two minutes. Data points were usually plotted as counts per two-minute periods.

(b) *Determination of Pentazocine* — Pentazocine levels in plasma and urine were determined as described by Tobin and Miller (1978).

(c) *Receptor Isolation* — Brain tissue was removed from freshly sacrificed animals. The desired portion was cut out, weighed and homogenized in 50 ml ice-cold tris buffer (pH 7.4) with a Ten-Broeck tissue grinder. The homogenate was centrifuged at 27,000 g for 15 minutes at 2°C. The supernatant was discarded; the pellet was resuspended in fresh ice-cold buffer and centrifuged as above. The final suspension was diluted to 105.6 x tissue weight and used in the binding assay.

(d) *Binding Assay* — Polystyrene tubes containing 100 μl of varying concentrations of unlabelled drug and 1.9 ml of the receptor homogenate were incubated for five minutes at 37°C in a water bath. The tubes then were cooled in an ice-water bath for one minute and the required amount of (^{3}H) morphine was added. Following a 15-minute incubation at 37°C, the reaction was stopped by adding 4 ml ice-cold tris buffer. The tubes were centrifuged at 3,000 g for 10 minutes at 2° C and the supernatant was discarded. The pellet, suspended in 400 μl of 10 *per cent* sodium dodecyl sulfate, was transferred to a glass vial and counted in 5 ml of Research Products 3a70 liquid scintillation cocktail in a Beckman LS-230 counter.

(e) *Chemicals and Reagents* — Authentic pentazocine base, used to prepare standards, was from Sigma Chemical Co., St. Louis.

Pentazocine was used experimentally as injectable Talwin^{R}, 30 mg/ml, from Winthrop Laboratories, Sterling Drug Co., Inc., New York. Liver β glucuronidase was from Sigma Chemical Co. Fentanyl citrate was a kind gift of McNeil Laboratories, Fort Washington, PA. All solvents used were of nanograde purity. All glassware used in these experiments was silanized in a one *per cent* solution of dichlorodimethylsilane (Aldrich Chemical Co.) in toluene.

Results

Figure 1 shows plasma levels of pentazocine after rapid intravenous injection of 1 mg/kg pentazocine in four horses. Plasma levels of pentazocine fell relatively rapidly for the first two hours, then more slowly, with an apparent terminal half-life of about 138 minutes from a linear regression analysis of all points from 2.5 hours on. This regression line extrapolated back to a zero time intercept (B) of 103 ng/ml. By curve peeling the half-time for the α or distribution phase was determined to be 27 minutes and the zero time intercept for this phase (A) was 365 ng/ml. From these data and the equations describing a two-compartment open model, the pharmacokinetic parameters describing a two-compartment open model derived from and fitting the data of Fig. 5 were calculated.

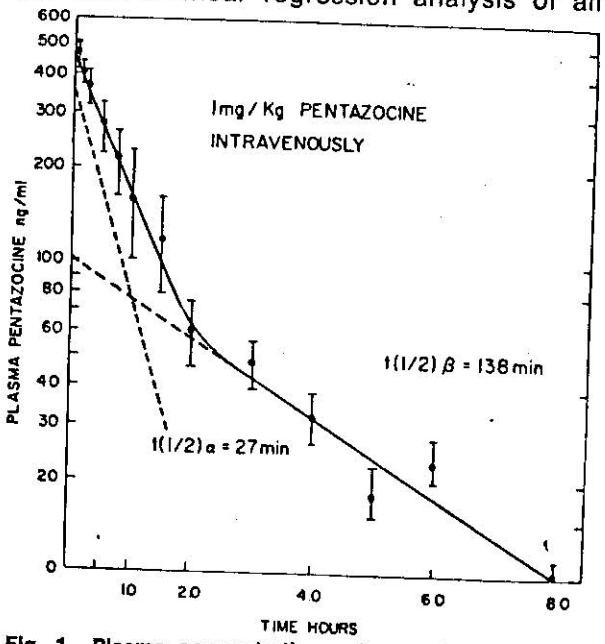

Fig. 1—Plasma concentrations after rapid intravenous injection of 1 mg/kg pentazocine.

The solid circles (●—●) show plasma concentrations of pentazocine after rapid IV injection of 1 mg/kg pentazocine. The β phase half-life was determined by fitting a linear regression to all data points from two hours on, and is represented by the solid line from two hours on. The α phase half-life was determined by deducting the projected β phase from all data points prior to two hours. All data points are the means ± standard errors of the means of determination on at least four different horses. *(Reprinted by permission of The Journal of Equine Medicine & Surgery).*

Figure 2 shows urinary concentrations of pentazocine following intravenous injection of 1 mg/kg. Urinary concentrations of pentazocine declined rapidly at first, then more slowly, to yield an eventual urinary half-life of about 8 hours over the following four days. Pentazocine was detectable in equine urine for up to 100 hours after its intravenous administration.

Figure 3 shows plasma levels of pentazocine after intramuscular injection of 0.66 mg/kg. Plasma levels of the drug rose slowly, peaked at about 30 minutes and then de-

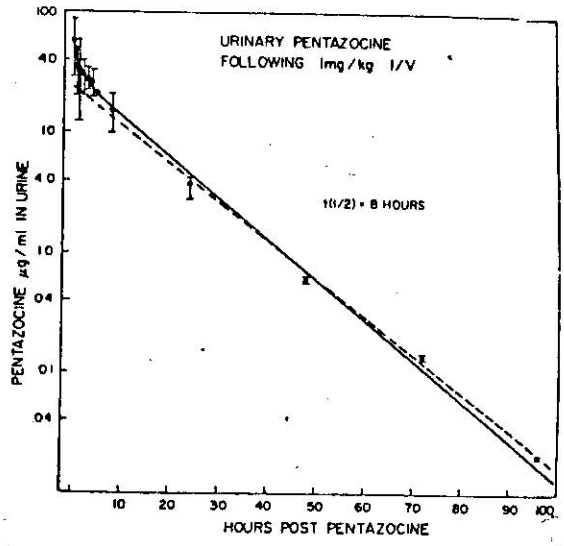

Fig. 2—Urinary concentrations of pentazocine following 1 mg/kg intravenously.

The solid circles (●—●) show urinary concentrations of pentazocine following 1 mg/kg IV. The solid line was fitted to the data points by eye. The dotted line represents a least squares regression fit to all the points from 10 hours on. All points are the means ± the standard errors of the means of determination on at least four different horses. *(Reprinted by permission of The Journal of Equine Medicine & Surgery).*

clined. This decline was relatively rapid at first with a half-life of about one hour, but the later decline was slow with a half-life of about eight hours in the terminal phase. Comparison of the areas under the curves suggested that pentazocine was essentially completely absorbed after intramuscular injection.

Figure 4 shows binding of (^{3}H) morphine to homogenates of rat cerebral tissue in the presence of excess unlabelled morphine. In the presence of excess unlabelled morphine binding was somewhat less than that observed in the absence of added unlabelled morphine. This suggests the presence of a population of receptors from which (^{3}H) morphine was excluded by the addition of excess unlabelled morphine.

Deduction of the non-saturable from the total binding yielded a compartment of binding which followed Michaelis-Menten kinetics and is thought to represent binding to the opiate receptor. Other work has shown that binding to this pool can be specifically prevented by opiate antagonists or by drugs which have no narcotic activity (Pert and Snyder, 1973). The inset shows a linear transformation of the saturable binding data, suggesting a population of narcotic receptor sites with an affinity for (^{3}H) morphine of about 2.5 x 10^{-8}M.

Figure 5 shows the distribution of this same population of binding sites in equine brain tissue with relatively low binding in the cerebellum and pons, and about three times greater binding in the cerebrum and thalamus. The data suggest the presence of the same opiate specific receptors in equine tissue with relatively greater receptor concentrations in the thalamus than is found in other species.

Figure 6 shows an attempt to use this specific binding of narcotic drugs to the opiate receptor as an assay for the presence of narcotic drugs in the urine of horses. (^{3}H) morphine was allowed to bind to the narcotic receptor in the presence and absence of different concentrations of fentanyl, added either in aqueous solution or spiked into equine

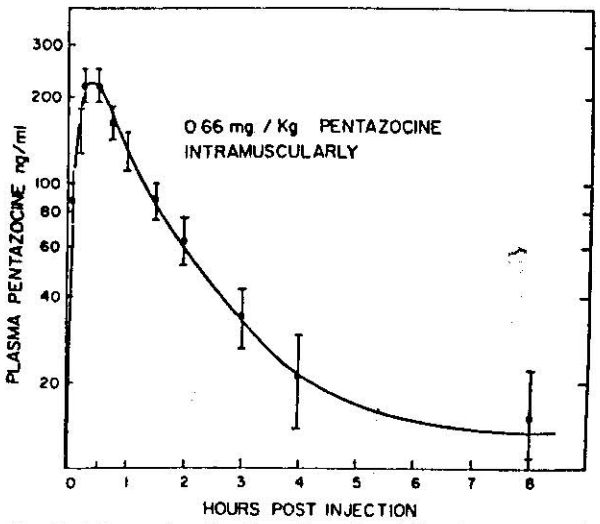

Fig. 3—Plasma levels of pentazocine following 0.66 mg/kg intramuscularly.

The solid circles (●—●) show plasma levels of pentazocine after 0.66 mg/kg pentazocine was administered by deep intramuscular injection. All data points are means ± the standard errors of the means of determinations on four different horses. *(Reprinted by permission of The Journal of Equine Medicine & Surgery).*

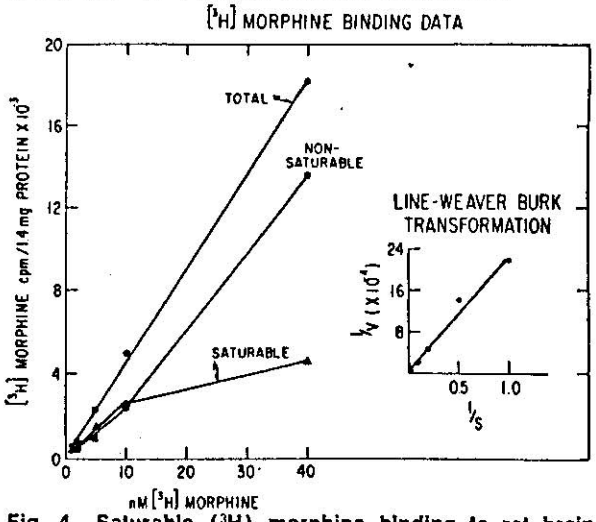

Fig. 4—Saturable (^{3}H) morphine binding to rat brain homogenate.

Rat cerebrum homogenate was prepared and (^{3}H) morphine binding measured as outlined in METHODS. The solid circles (●—●) show binding of the indicated concentrations of (^{3}H) morphine to this tissue in the absence of added unlabelled morphine. The solid squares (■—) show binding in the presence of excess (10^{-4} M) unlabelled morphine. The solid triangles (▲—▲) show that portion of binding sensitive to the presence of excess unlabelled morphine, i.e. the saturable binding. The inset shows a Lineweaver-Burk transformation of the saturable (^{3}H) morphine binding data. From this transformation the apparent affinity of (^{3}H) morphine for its specific binding sites is about 2.5 x 10^{-8} M.

urine. When added in aqueous solution, concentrations of fentanyl of less than 10^{-9}M produced a log-linear displacement of (^{3}H) morphine binding from the narcotic receptor. However, equine urine alone produced a 50 *per cent* displacement of (^{3}H) morphine from the narcotic receptor and the response to fentanyl "spiked" into equine urine was poor. The data suggest that equine urine contains material, possibly endogenous opiates which cause displacement of (^{3}H) morphine from the narcotic receptor and which appears to interfere with actions of fentanyl to displace (^{3}H) morphine. Similar results were observed with a number of other equine urines and this particular approach to the assay of fentanyl was abandoned.

While data on blood and urinary levels of drugs are useful *per se*, the ability to correlate this data with pharmacological effects is very useful in the equine forensic area. One of the principal pharmacological actions of narcotic analgesics in the horse is to stimulate motor activity, apparently via a dopaminergic action (Lal, 1978). Observing the motor response to fentanyl it occurred to us that simply counting footsteps might be a useful way of quantitating this response to the narcotic analgesics. As outlined in METHODS, these experiments were performed by wrapping the left foreleg in a white bandage and scoring the animal for one count every time this foot left the ground. During a control period, animals showed an activity in the order of about 10 footsteps/

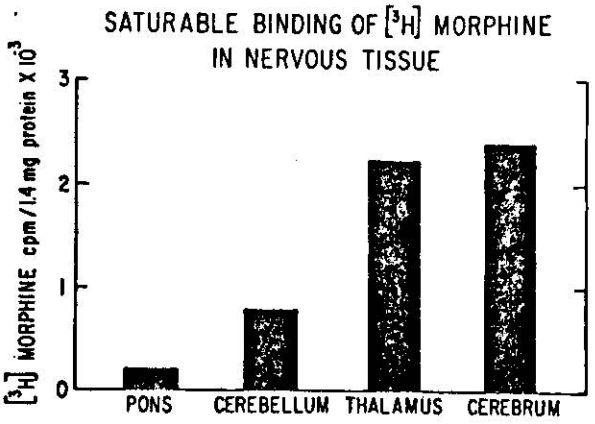

Fig. 5—Saturable binding of (^{3}H) morphine in equine nervous tissue.

The indicated areas of horse brain were prepared and assayed for binding as outlined in METHODS. Total binding was determined using 10 nM (^{3}H) morphine in the absence of unlabelled drug. Simultaneously, non-saturable binding was determined by including excess unlabelled morphine (10^{-4} M) with the 10 nM (^{3}H) morphine. The vertical bars show the relative amounts of saturable (^{3}H) morphine binding observed in tissue from the indicated areas.

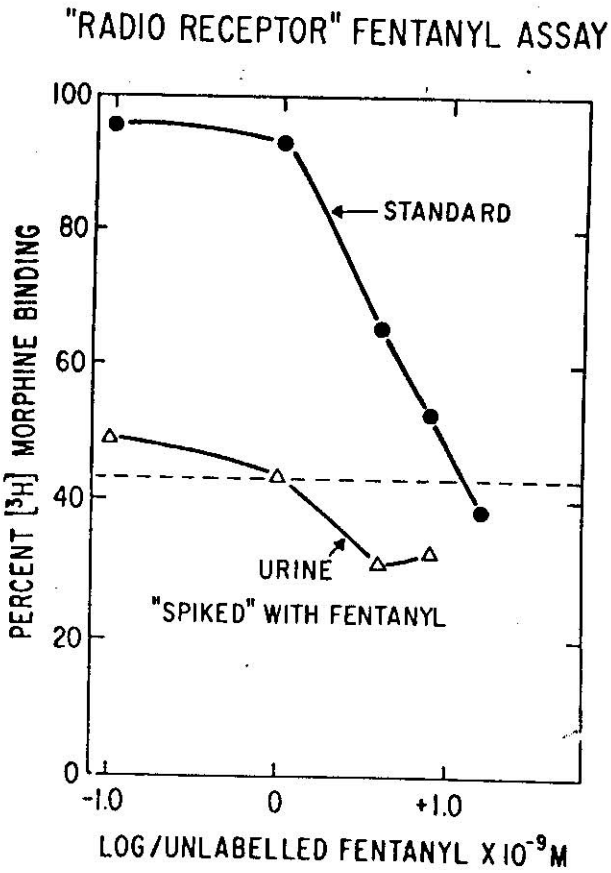

Fig. 6—"Radio-receptor" fentanyl assay.

20 ml horse urine samples spiked with 0.1, 1, 4 and 8 nM fentanyl were adjusted to an alkaline pH with 4 ml saturated sodium tetraborate buffer. The fentanyl was extracted into 4 ml ethyl acetate by rotoracking on low speed for 30 seconds. After centrifugation, the ethyl acetate layer was separated off, evaporated to dryness and reconstituted with 0.5 ml water. Fentanyl standards and control and spiked urine extracts were assayed using 10nM (^{3}H) morphine and rat cerebrum homogenate as outlined in METHODS. The circles (●—●) show displacement of (^{3}H) morphine from the opiate receptor by the fentanyl standards; the triangles (Δ—Δ) show displacement by the fentanyl spiked urines; and the dashed line shows displacement by extracts of control urine. The data points are from a single experiment and are typical of a number of assays.

two-minute period (Fig. 7). After rapid intravenous injection of 1 mg fentanyl citrate into a 1,000 lb horse, spontaneous activity initially increased to about five times this rate and then decayed away to control levels by about 20 minutes. If the dose was increased to 5 mg the initial response was markedly increased, peaking at about 140 steps/2 minutes, then decaying away in parallel with the first response to disappear at about 60 minutes post-injection. Doubling the dose of fentanyl to 10 mg produced only a small increase in peak motor activity, which again declined in parallel with the previous response and had returned to control by about 70 minutes. Thus, this simple method allowed what appears to be very accurate and precise quantitation of one of the pharmacological effects of the narcotic analgesics in the horse.

Figure 8 shows the results obtained when this same method was used to quantitate the motor response to pentazocine in the horse. Saline injection or low doses of pentazocine

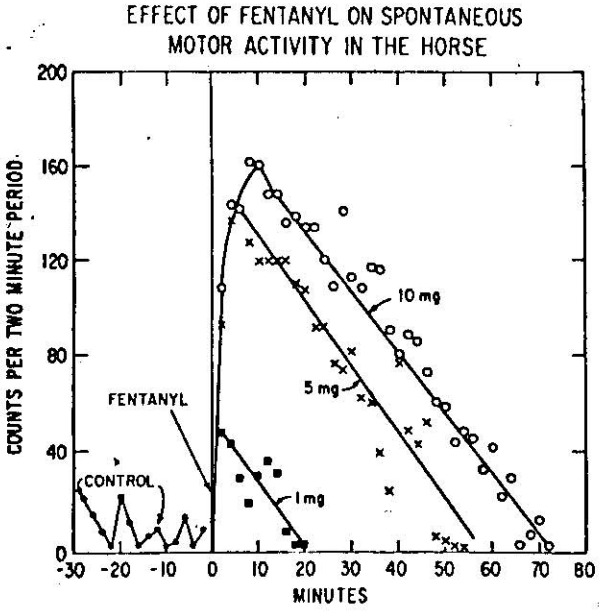

Fig. 7—Effect of fentanyl on spontaneous motor activity in the horse.

The solid circles (●—●) represent spontaneous motor activity in the horse prior to dosing with fentanyl, determined as outlined in METHODS. The squares (■—■) show counts following an IV injection of 1 mg fentanyl; crosses (X—X) after 5 mg fentanyl, and open circles (O—O) after 10 mg fentanyl.

intravenously (up to 0.5 mg/kg) produced little change in spontaneous motor activity in this and other horses. A dose of 1.0 mg/kg produced a modest increase in motor activity for the first 20 minutes or so after its administration, which rapidly declined toward control levels. However, a dose of 2.0 mg/kg produced a marked increase in spontaneous motor activity, the effect of which peaked at about 50 minutes after dosing and was still apparent about two hours later.

Discussion

When administered intravenously, plasma levels of pentazocine peaked at about 500 mg/kg and then declined, rapidly at first, and then more slowly, with an apparent terminal or β phase half-life of about 138 minutes. When given intramuscularly, plasma levels of pentazocine similarly declined rapidly at first, then more slowly, and the terminal half-life of the drug when given by this route was not determined. By both routes it appeared that the terminal half-life of the drug was relatively long.

This apparently long plasma half-life of pentazocine showed up in an unusually long urinary "clearance time" for this drug, the principal glucuronide metabolite of pentazocine being found in urine for up to five days after dosing with the drug. This is an unusually long clearance time for any drug in the horse and horsemen and veterinarians should be aware of this prolonged "clearance time" when they treat racing horses with pentazocine.

The recent identification of the narcotic receptor in vertebrate nervous tissue suggested to us that it might be possible to use this specific receptor as part of a displacement type assay for narcotic drugs in horse urine. As shown in Figs. 4 and 5, it is not difficult to isolate and identify this receptor material in both rat and equine nervous tissue. However, it appears that equine urine contains large amounts of endogenous materials which cross-react with the opiate receptor and

both displaces (^{3}H) morphine from this receptor and interferes with the actions of fentanyl on (^{3}H) morphine binding. The nature of these materials is unknown, but the possibility exists that they may be endogenous opiate peptides. If equine urine does indeed contain such opiate peptides these may well interfere with any radio-immunoassay for narcotic analgesics in equine urine.

Quantitation of pharmacological response to drugs in the horse has long been a problem. Studies on the performance effects of drugs using Thoroughbreds and Standardbreds under racing conditions have, in general, led to inconclusive results. Further, if such studies must be carried out it is important to accurately define dose response and time response relationships for the drugs to be tested so that expensive performance trials are carried out with the proper dose and at the optimum time post-dosing. Figure 7 shows that the simple step counting mechanism gave excellent results with the narcotic fentanyl, allowing very accurate quantitation of both dose and time response to this drug.

In another series of experiments, the actions of fentanyl on reserpinized horses was tested. It turned out that the primary action of reserpine in an experiment such as Fig. 7 was to reduce the "background" spontaneous movement but that it did not affect or reduce in magnitude the clear-cut response to fentanyl itself. Thus, it seems likely that the pretreatment with reserpine will act to reduce background activity only and may allow very accurate definition of the actions on motor activity of small doses of narcotic drugs or the actions of mixed agonists such as pentazocine.

Figure 8 shows the pharmacological actions of pentazocine in the same test system as was used for fentanyl in Fig. 7. At doses up to 0.5 mg/kg, pentazocine produced little change in spontaneous motor activity from the control. A dose of 1.0 mg/kg produced a marked initial increase in motor activity which rapidly decayed away to control levels. Only the 2.0 mg/kg dose produced a substantial increase in motor activity which peaked at about 50 minutes and then decayed slowly over the next hour. If the hypothesis that effective occupation of the narcotic receptors is required for both the motor response to pentazocine and its analgesic effects is accepted, one might conclude from this data that 2.0 mg/kg of pentazocine in the horse is required for a good analgesic effect and that the effect should be limited to about two hours. Almost exactly similar conclusions about the dose response effect for analgesia due to pentazocine and its duration of action in the horse have been reported by Lowe using a colic model in ponies (Lowe, 1969, 1978).

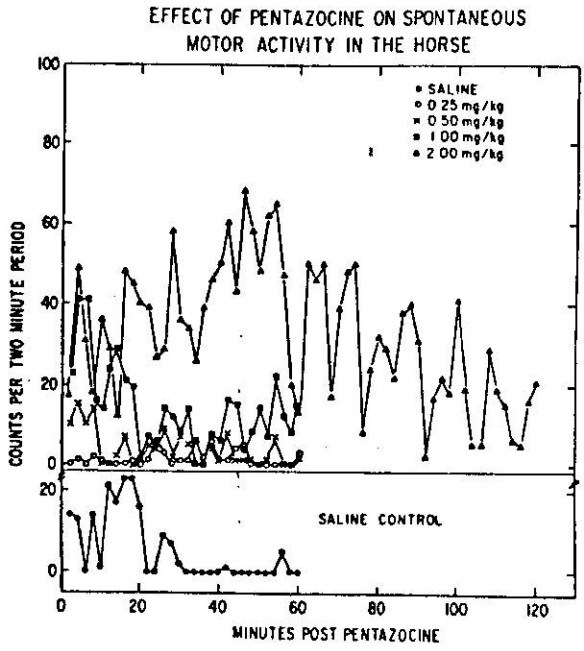

Fig. 8—Effect of pentazocine on spontaneous motor activity in the horse.

The horse was injected with saline and then increasing amounts of pentazocine. The number of times the horse lifted its left front foot in a 2-minute period was recorded as an indication of motor activity. Activity following IV saline injection is shown by closed circles (●—●); 0.25 mg/kg by open circles (O—O); 0.5 mg/kg by crosses (X—X); 1.0 mg/kg by squares (■—■); and 2.0 mg/kg by triangles (Δ—Δ).

References

Davis, Lloyd E. and Sturm, Bonnie L. (1970). Drug effects and plasma concentrations of pentazocine in domestic animals. Am. J. vet. Res. **31**, 1631-1635.

Lal, Harbens. (1978). Narcotic dependence, narcotic action and dopamine receptors. Life Sci. 17, 483-496.

Lowe, J. E. (1969). Pentazocine (Talwin-Vit) for the relief of abdominal pain in ponies — a comparative evaluation with description of a colic model for evaluation of analgesia. Proc. AAEP. 31-46.

Lowe, J. (1978). Rompun, Talwin, Demerol and Novin for relief of a balloon-induced colic : A double blind comparative evaluation. J. Eq. Med. Surg. In press.

Miller, J. R., Roberts, B. L., Blake, J. W., Valentine, R. W. and Tobin, T. (1977). Drug interactions in the horse. III. Effects of furosemide on urinary concentrations of glucuronide metabolites of pentazocine. Res. Commun. Chem. Pathol. Pharmacol. 17, 447-456.

Pert, C. B. and Synder, S. H. (1973). Opiate receptor; demonstration in nervous tissue. Science. 179, 1011-1019.

Remington's Pharmaceutical Sciences, J. E. Hoover, ed., 15th ed., 1975. Mark Publishing Co., Easton, PA.

Tobin, T., Blake, J. W., Sturma, L., 'Arnett, S. and Truelove, J. (1977). Pharmacokinetics and behavioural effects. Am. J. vet. Res. 38(5), 637-47.

Tobin, T. (1978). Narcotic analgesics and the opiate receptor in the horse. J. Eq. Med. Surg. 2, 397-399.

Tobin, T. and Miller, J. R. (1978). The pharmacology of narcotic analgesics in the horse. I. The detection, pharmacokinetics and urinary "clearance time" of pentazocine. J. Eq. Med. Surg. 3, 191-198.

RECOMMENDATIONS BY LIAISON COMMITTEE ON EQUINE DISEASES FOR THE PREVENTION AND CONTROL OF CONTAGIOUS EQUINE METRITIS IN THE 1980 SEASON

Introduction

1. The Code as recommended for use in 1979 has been revised in the light of experience for the 1980 season. The improvement in the CEM situation was maintained in the 1979 season and this, it is felt, was largely due to the extent with which these recommendations were implemented by the bloodstock industry.

2. Review of Contagious Equine Metritis (CEM) from September 1978 to September 1979

During the period under review there was a decline in the prevalence and distribution of CEM as compared with the same period in 1977-1978. Fewer carrier mares were detected in the interbreeding season and only one outbreak of disease was confirmed.

A total of 17,630 sets of swabs were examined at the eighteen approved laboratories— 16,655 from mares and 975 from stallions. *Haemophilus equigenitalis* was isolated from 28 thoroughbred horses. There were no isolations from non-thoroughbreds. The one outbreak began with the covering of a carrier mare at the end of March, 1979. A group of 15 mares was found to be infected, of which two showed clinical signs. Only one of the 15 mares was subsequently confirmed in foal.

3. Blood Tests

It has been decided to discontinue blood sampling of high risk mares for serological examination except in the event of an outbreak when all mares covered by the affected stallion will be blood tested 19-40 days after service.

CODE OF PRACTICE

4. Recommendations to Mare and Stallion Owners for the 1980 Covering Season

The recommendations set out below are made in the light of research work and practical experience gained during the last three breeding seasons. *The recommendations are the minimum requirements for the examination of mares and stallions and it is emphasised that owners should consult their veterinary advisers concerning the implementation of the code.* As more information becomes available amendments to the code may be introduced.

5. DEFINITIONS

Mares have been divided into two categories (1) high risk and (2) low risk —

(1) **High Risk Mares**

(a) Mares from which the CEM Organism, *Haemophilus equigenitalis* has been isolated;