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IMMUNOASSAY DETECTION OF DRUGS IN HORSES
I. PARTICLE CONCENTRATION FLUOROIMMUNOASSAY DETECTION OF FENTANYL AND ITS CONGENERS

John McDonald, Roy Gall, Paul Wiedenbach, Vivian D. Bass, and B. DeLeon
Illinois Racing Board Laboratory
750 South State Street
EMCH Mendel Building
Elgin, Illinois 60120
and
Catherine Brockus, Diana Stobert, Siong Wie and Charles A. Prange
International Diagnostics Systems Corporation
2614 Niles Avenue
P.O. Box 799
St. Joseph, Michigan 49085
and
J.-M. Yang, C.L. Tai, T.J. Weckman, W.E. Woods, H.-H. Tai, J.W. Blake, and T. Tobin
Kentucky Equine Drug Research and Testing Programs
Department of Veterinary Science and School of Pharmacy
University of Kentucky
Lexington, Kentucky 40546-0076

SUMMARY

We investigated the use of particle concentration fluorescence immunoassay (PCFIA) as a technique for drug detection in racing horses. The test was constructed from an antiserum to a carboxyfentanyl-BSA conjugate and carboxyfentanyl linked to

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Correspondence should be addressed to Dr. Tobin

b-Phycoerythrin. Using these reagents and a PCFIA apparatus levels of fentanyl as low as 0.1 ng/ml could be detected by the assay. In addition, cross-reactivity studies on this assay showed that the anti-serum cross-reacted well with carfentanil, sufentanil and the methylated analogs of fentanyl. We therefore evaluated the ability of these agents to produce pharmacological effects in the horse and the ability of this test to detect pharmacologically significant doses of this drug in racing horses.

All of these agents produced good locomotor responses in horses at doses of between 0.1 and 10 ug/kg. Of these agents, carfentanyl was the most potent followed by 3-methylfentanyl, sufentanyl, α-methylfentanyl, and fentanyl. Similarly, when these agents were administered to horses at doses sufficient to produce a pharmacological response, all produced sufficient inhibition of fluorescence in the PCFIA system to enable their detection in post-race urines from these horses.

Since PCFIA is a much faster technique than radioimmunoassay, is of approximately similar sensitivity, and requires much less instrumentation we concluded that this technique holds considerable promise as an equine drug testing technique.

INTRODUCTION

Fentanyl (N-phenyl-N-[1-(2-phenethyl)-4-piperidinyl]propanamide) is a synthetic opioid derivative of meperidine and a narcotic analgesic with about 80 to 150 times the potency of morphine (Tobin, et al., 1979; Jaffe, Martin, 1985). Its narcotic actions are characterized by rapid onset and short duration of action. The

pharmacologic actions are similar to those of morphine, and fentanyl is considered a pure u-opioid agonist (Martin, 1984).

The pharmacological actions of fentanyl in the horse are different from its actions in man. In horses, fentanyls produce marked locomotor stimulation, along with their analgesic effects. The sum of these actions, in that they act to alleviate lameness and stimulate running, is quite likely to be useful in a racing horse. For these reasons the fentanyls have been widely used in racing horses, despite the fact that their use is uniformly illegal (Tobin, 1981).

Among the congeners of fentanyl that have recently become available are carfentanil, sufentanil and the so-called "designer" fentanyls (Janssen, 1985; Moore, et al., 1986). These "designer" agents include α-methylfentanyl [1-(1-methyl-2-phenethyl) -4-(N-propionylanilino) piperidine] and 3-methylfentanyl [3-methyl-1-(2-phenyethyl) -4-(N-propionylanilino)piperidine] which are produced illicitly. As fentanyl analogs, these agents have the potential to be used illegally in racing horses. However, no information is available as to the ability of currently used systems to detect abuse of these agents.

With regard to control of these agents in horse racing there is a particular need for rapid and sensitive detection methods for these agents that are adaptable to pre- and post-race drug screening. The method must be rapid since the time window for pre-race screening is less than four hours under most circumstances. The method must be sensitive because drugs that are abused in horse racing are difficult to detect in urine, and pre-race testing mandates that they be detected in blood, which requires very sensitive analytical methods.

The requirement for sensitivity makes immunoassay the method of choice. The requirement for speed rules out radioimmunoassay, which is too slow to be adaptable to pre-race testing. One recent approach to this problem is the particle concentration fluorescence immunoassay (PCFIA) technique developed by the Pandex Corporation (Jolley, et al., 1984; Jolley, 1983). In this approach the blood or urine sample is allowed to react with the antibody and the drug-fluorophore complex in a microtiter well (Fig 1). After an equilibration time of five to ten minutes, second antibody coated latex particles are added to the system (1) and the complex concentrated by vacuum the bottom of the microtiter well (2). The particles are then washed to remove unbound fluorescent material and the fluorescence response read (3). The intensity of the fluorescence is inversely related to the amount of free drug in the sample. Preliminary experiments suggested that this system is sufficiently fast and sensitive to be readily adaptable to pre- and post-race drug screening in horses.

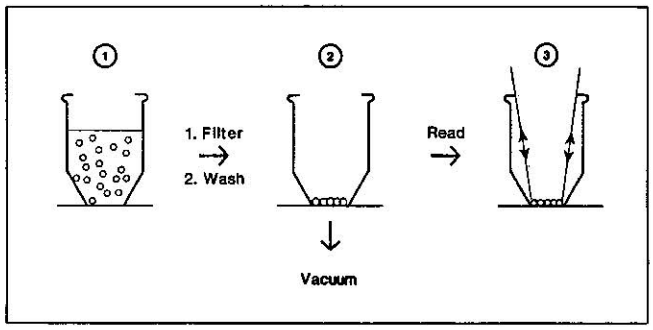

Fig 1 Principle of Particle Concentration Fluoroimmunoassay

Fentanyl is allowed to displace fentanyl-B-phycoerythrin from the anti-fentanyl plasma (1). Second antibody coated latex particles are added to the system and the particles drawn down by application of a vacuum (2). The fluorscence of the resulting layer of particles is then measured and the loss of fluorescence estimated (3). (Adapted from Jolley, 1983).

MATERIALS AND METHODS

Horses

Mature Thoroughbred, half Thoroughbred and Standardbred horses (400-600 kg) were used throughout. The animals were kept at pasture and allowed free access to food and water. The horses were placed in standard box stalls (17 sq M) approximately 12 hours prior to the experimental session for acclimatization.

Drugs

Carfentanil was obtained from a commercial supplier in an injectable solution (Wildnil®, 3 mg/ml, Wildlife Laboratories, Inc., Fort Collins, CO). Sufentanil was also obtained in an injectable form from a commercial source (Sufenta®, 50 ug/ml, Janssen Pharmaceutica Inc., Piscataway, NJ). 3-Methylfentanyl was dissolved in 10 ml of sterile physiological saline (pH 7.0). α-Methylfentanyl was dissolved by adding the crystalline form to 10 ml of sterile physiological saline (pH 7.0) and heating to 58° C with stirring. Drug administration was by rapid injection into the left jugular vein. These methylfentanyl analogs were generously supplied by Dr. R. L. Hawks of the National Institute of Drug Abuse, Rockville, MD. Urine samples were collected by bladder catheterization, and were stored frozen until assayed. All urine samples were filtered through Spin-X® microcentrifuge filters (Costar®, Cambridge, MA) prior to assay.

Locomotor Assay Locomotor Studies

For this measurement horses were placed in box stalls which are enclosed on all sides. A window made of one-way mirrored glass located in each door permitted observers to record behavior without detection by the animal. Locomotor behavior was quantified by

counting the number of footsteps taken per 2 min period. A footstep was scored each time the right foreleg was lifted off the ground and returned along with a positional change (Combie, et al., 1979). Locomotor assays with carfentanil were based on doses of 0.08, 0.14, 0.20, 0.30 and 0.60 ug/kg. Sufentanil was administered at doses of 0.25, 0.50, 1.00, 1.33 ug/kg. Preliminary studies with 3-methylfentanyl indicated that intravenous doses at 4 ug/kg were in excess of tolerable limits. This dose produced severe excitement, tachycardia and tachypnea in one horse. This dose was antagonized with 8mg naloxone i.m. (Narcan, DuPont Pharmaceuticals Inc., Manati, Puerto Rico). Subsequent doses of 0.4, 0.7, and 1.0 ug/kg 3-methylfentanyl were well tolerated in all subjects. These subsequent three dose levels formed the basis of the 3-methylfentanyl locomotor assay. α-Methylfentanyl was administered at doses of 1.0, 2.0, 4.0, 8.0, and 13.0 ug/kg and was well tolerated by all subjects.

Locomotor activity was quantified for 16 min prior to each injection to establish the pretreatment baseline. Footstep frequency was then recorded every 2 min for a minimum of 60 min post injection.

Particle Concentration Fluoroimmunoassay

The basic functional unit in the Pandex PCFIA is a 96 well plate with a filter base in each plate. To each well is added 20 µl of fentanyl-β-phycoerythrin (Fentanyl-BPE) prepared as described by Wie et al. (1982), 40 µl of anti-fentanyl antibody, and 40 µl of blank, standard, or test sample. The system is allowed to equilibrate for about 10 minutes when a second antibody system is added. The second antibody consists of goat anti-rabbit antibody bound to latex beads. The system is allowed to react for another 10 minutes and then the

fluid is drawn out of the system through the filter membrane. The reaction system is then washed with about 80 μ liters of phosphate buffer to resuspend the particles, and the system again drawn down with the vacuum. The filtration step has the effect of concentrating the latex beads 1000-fold, thereby increasing the sensitivity of the method (Fig 1). After the wash step, the fluorescence of the particles at 545 and 575 nm is measured. The mean response from control urines is usually about 25,000-30,000 arbitrary fluorescence units/well.

RESULTS

The relative potencies of fentanyl and its congeners as locomotor stimulants in the horse are presented in Fig. 2. Carfentanil is the most potent locomotor stimulant, with an ED50 for locomotor response in the horse of about 0.5 ug/kg. Sufentanil and 3-methylfentanyl are about half as potent as carfentanil, with an ED50 for a locomotor response in the horse of about 1.0 ug/kg. Fentanyl and α-methylfentanyl are the least potent members of the group with an ED50 for locomotor stimulation of about 10ug/kg. Nevertheless, all of these drugs produce their pharmacological effects at doses of 1 mg/horse or less. To date, we are aware of no useful detection method for carfentanil.

Figure 3 shows a standard curve for the displacement of Fentanyl-BPE from the binding system by fentanyl. The threshold of sensitivity of the test is in the order 100 picograms/ml with an I-50 for displacement of about 2.0 ng/ml. Given these sensitivities this method should be capable of detecting fentanyl administration to horses, and at least some of the other congeners of fentanyl depending on the sensitivity of the assay to each specific congener.

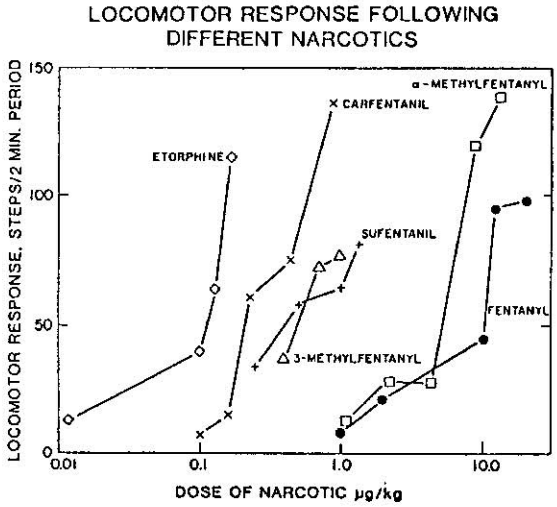

Fig 2 Locomotor responses following different narcotic analgesics in the horse

The symbols show the locomotor responses to the indicated doses of etorphine (◇) (Combie et al., 1979), carfentanil (×), 3-methylfentanyl (△), sufentanil (+), α-methylfentanyl (□) and fentanyl (●) in racehorses.

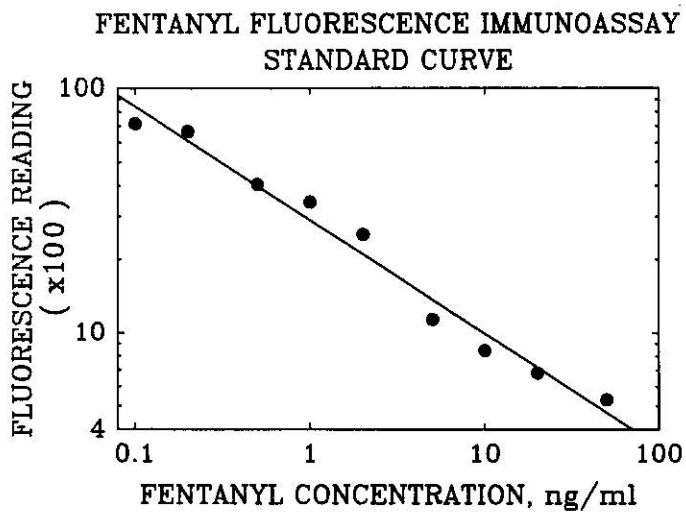

Fig 3 Fentanyl fluorescence immunoassay standard curve

The solid circles (●) show the displacement of fentanyl-BPE from the anti-fentanyl antibody by addition of the indicated concentration of fentanyl.

Table I shows the ability of the other congeners of fentanyl to displace Fentanyl-BPE in the PCFIA test. As in the previous test fentanyl displaced Fentanyl-BPE with an I-50 of about 2 ng/ml. Carfentanil, thienylfentanil, -methylfentanyl, p-fluorofentanil and 3-methylfentanyl all displaced Fentanyl-BPE from the antibody approximately as effectively as fentanyl. The other fentanyls tested did not effectively displace Fentanyl-BPE, and neither did furosemide, phenylbutazone, phenothiazine, morphine, or cocaine at concentrations of 10 ug/ml or greater.

Table II shows the ability of this test to detect fentanyl, carfentanil and sufentanil after their administration to horses. Horses were dosed with 100 ug of fentanyl i.v. and also with 100 and 125 ug of carfentanil and with 1 mg of sufentanil. Any inhibition of Fentanyl-BPE binding greater than 20% was considered a presumptive "positive". The samples were collected, randomized as indicated and presented as unknowns to the analyst. As shown in Table II all of the drug administrations were readily detected.

Two congeners of fentanyl that are reportedly available on the illicit market are -methylfentanyl and 3-methylfentanyl. These agents are approximately equivalent in potency with fentanyl and also have the ability to stimulate a locomotor response in the horse. As such, they are likely candidates for illegal use in racing horses. As shown in Tables III and IV these agents were also readily detected in horse urine by use of this PCFIA test.

For any test to be useful as a screening test, there should be a high probability that a "presumptive positive" developed by the test is in fact a real positive. To enable this assignment to be made with

confidence, one needs to know the range of fluorescence seen in normal urines, and particularly the range of inhibition in these urine samples. As shown in Fig 4, determination of the range of fluorescence values in 54 post-race urines from horses racing in Kentucky showed inhibition of up to 15% in these urine values. Based on this range and the relatively small number of horses tested, assignment of a presumptive positive when the inhibition observed is greater than 20% would appear to yield a high probablity of detecting true positives.

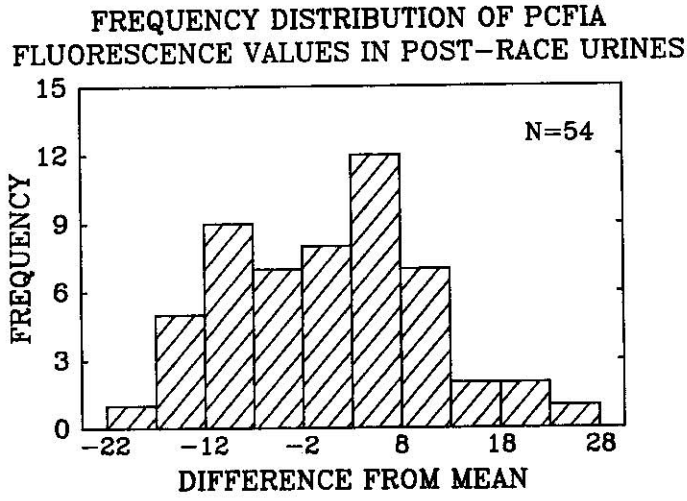

Fig 4 Frequency Distribution of Fluorescence Values in Post-Race Urines.

The bars show a frequency distribution of the PCFIA values observed in 54 post-race urines from horses racing in Kentucky. The negative values represent fluorescence values of less than the mean value (inhibition) while the positive values represent stimulation of fluorescence.

TABLE I

DISPLACEMENT OF FENTANYL-BPE BY FENTANYL ANALOGS

Drug level ng/ml	Fentanyl	Carfentanil	Thienyl Fentanyl	-methyl Fentanyl	3-methyl Fentanyl	pfluoro Fentanyl	Sufentanil	Lofentanil	Alfentanil
0	25,900	25,900	25,900	25,900	25,900	25,900	25,900	25,900	25,900
0.1	22,681	23,172	22,245	23,174	23,447	22,415	24,152	25,376	25,124
0.5	13,252	18,423	--	--	--	--	--	--	--
1.0	8,650	15,965	10,497	15,822	13,627	10,058	23,357	24,255	24,980
5	3,035	9,967	--	--	--	--	--	--	--
10	2,330	8,816	2,692	9,472	4,515	2,735	20,853	22,427	25,010

The columns show the displacement of Fentanyl-BPE from fentanyl antiplasma by the increasing concentrations of the medicated congeners of fentanyl. No significant displacement was observed when concentrations of 10 ug/ml or more of furosemide, phenylbutazone, phenothiazine, morphine or cocaine were added to the system.

TABLE II

DETECTION OF FENTANYL, CARFENTANIL AND SUFENTANIL IN EQUINE URINES

Sample #	Treatment	Fluorescence	% Inhibition	Status
1A	Fentanyl 100 ug i.v. 1-2 hour	793	97%	+
2A	Saline	23,626	8%	-
3A	Saline	26,508	-	-
4A	Carfentanil 125 ug i.v. 0-1 hour	17,154	33%	+
5A	Carfentanil 100 ug i.v. 0-1 hour	18,331	28%	+
6A	Sufentanil 1 mg i.v. 0-1 hour	13,491	47%	+
7A	Carfentanil 100 ug i.v. 4-6 hour	18,244	29%	+
8A	Sufentanil 100 ug 0-1 hour	16,303	36%	+
9A	Carfentanil 125 ug i.v. 1-2 hour	15,524	39%	+
10A	Carfentanil 160 ug i.v. 2-4 hour	16,548	35%	+
11A	Carfentanil 160 ug i.v. 1-2 hour	11,997	53%	+
12A	Saline	18,290	28%	+
13A	Fentanyl 100 ug i.v. 2-4 hour	1,252	95%	+
14A	Carfentanil 100 ug i.v. 2-4 hour	18,212	29%	+
15A	Saline	31,053	-	-
16A	Carfentanil 125 ug i.v. 2-4 hour	16,838	34%	+
17A	Carfentanil 160 ug i.v. 0-1 hour	17,503	32%	+
18A	Saline	26,182	-	-
19A	Carfentanil 160 ug i.v. 4-6 hour	18,784	27%	+
20A	Fentanyl 50 ug i.v. 1-2 hour	4,055	84%	+
	Control Fluorescence	25,586	0%	-

All samples are urine from horses to whom the indicated drugs were administered. Urine samples were drawn at the indicated times, filtered, and diluted 1:2 with distilled water. Any sample giving a greater than 20% inhibition of binding was considered positive. Sample #12A also yielded a positive although this was supposedly a negative sample.

DISCUSSION

Fentanyl has been widely used in racing horses for its analgesic and stimulant actions. This combination of pharmacological effects is likely to be particularly useful in a racing horse, and the narcotic analgesics have a long history of illicit use in racing. There is, therefore, a continuing need for good testing methods which allow detection of high potency drugs in racing horses. The ideal method is swift, sensitive, and inexpensive. In this communication we report

TABLE III

DETECTION OF 3-METHYLFENTANYL IN HORSE URINE

Sample	Treatment	Fluorescence	% Inhibition	Status	n
1	Control	27,453	--	-	6
2	3-Methfent 0.1	26,149	4.8%	-	3
3	3-Methfent 1.0	21,600	21.4%	-	3
4	3-Methfent 10.0	16,838	38.9%	-	3
5	3-Methfent 100.0	8,879	68.0%	-	3
6	Negative urine	27,580	104%	Neg	3
7	Negative urine	27,172	1.0%	Neg	3
8	Negative urine	27,645	--	Neg	3
9	3-Methfent, 2hrs	9,066	67.4%	Pos	3
10	3-Methfent, 4 hrs	2,517	91.3%	Pos	3
11	Negative	27,345	0.4%	Neg	3
12	3-Methfent, 2 hrs	9,817	64.6%	Pos	3
13	3-Methfent, 4 hrs	10,952	60.4%	Pos	3
14	Negative urine	24,979	9.1%	Neg	3
15	Negative urine	26,488	3.5%	Neg	3
16	Fent, 2 hrs	4,064	85.7%	Pos	3

Samples 1-5 represent control and samples to which the indicated concentrations of 3-methfentanyl were added. Samples 6, 7, 8, 11, 14 and 15 represent urine samples from untreated horses. Samples 9, 10, 12 and 13 represent samples taken at 2 and 4 hours from a horse treated with 0.5 mg/horse of 3-methylfentanyl. Sample 16 represents a sample from a horse treated 2 hours earlier with fentanyl. All samples showing more than 20% inhibition of binding were designated positive.

the capacity of Particle Concentration Fluorescent Immunoassay (PCFIA) to meet these requirements in testing for fentanyl and its congeners in horse urine.

The potency of these drugs is a particular problem since their concentrations in the blood and urine of horses is likely to be low. As shown in Fig. 1 , carfentanil is almost as potent as etorphine, the most potent narcotic analgesic studied in racing horses (Combie, et al., 1979). Similarly, sufentanil, a fentanyl analog now being introduced into human medicine is about 8 times more potent than fentanyl. 3-Methylfentanyl, an illicit or "designer" analog of

TABLE IV

DETECTION OF α-METHYL FENTANYL IN HORSE URINE

Sample	Treatment	Fluorescence	% Inhibition	Status	n
0	Control	27,458	0	-	6
1	Fent 0.2 ng/ml	24,577	10%	-	2
2	Fent 1.0 ng/ml	16,783	39%	Positive	2
3	Fent 5.0 ng/ml	4,863	82%	-	2
4	Fent 10.0 ng/ml	3,164	88%	-	2
5	Remake, Control	17,684	25%	Positive	2
6	α-Methfent, 2 hrs	4,013	77%	Positive	2
7	α-Methfent, 4 hrs	5,391	22%	Positive	2
8	T-3 Control	22,497	4%	Negative	2
9	T-3 + 2 hrs	3,507	85%	Positive	2
10	T-3 + 4 hrs	3,554	85%	Positive	2
11	T-4 Control	26,872	-14%	-	2
12	T-11 Control	20,897	11%	Negative	2
13	BEA Control	23,317	1%	Negative	2
14	Point Finale Control	22,605	4%	Negative	2
15	Y.S. Control	24,535	- 4%	Negative	2
16	Peanut Control	25,335	- 8%	Negative	2
17	Track Sample	24,485	- 4%	Negative	2
18	Track Sample	24,164	- 3%	Negative	2
19	Track Sample	23,229	1%	Negative	2
20	Track Sample	19,577	16%	Negative	2

Samples 1-4 represent controls (6) and samples to which the indicated concentrations of fentanyl were added. Samples 5-10 represent pre-drug and samples 2-4 hours post-dosing with 2 mg of α-methyl fentanyl. Samples 11-20 represent control samples from farm and track horses. All presumptive positives were calculated based on the fluorescence values observed in control urine samples, which averaged 23,499 fluorescence units.

fentanyl, is equipotent with sufentanil in eliciting a locomotor response, and α-methylfentanyl is about equipotent with fentanyl. Together these drugs form a group of agents with high abuse potential in racing horses and for which there is substantial difficulty in their detection.

On a theoretical basis the PCFIA method shows good potential for detection of these agents. The system has a theoretical limit of

sensitivity that is comparable to radioimmunoassay, (Jolley, 1983) and the actual testing process is much faster. Based on these characteristics, one might expect that the PCFIA technology would be superior to radioimmunoassay for routine pre and post-race urine testing.

On the other hand, there are a number of potential problems with this technology when it is applied to post race urine testing in horses. Horse urine contains unusually large quantities of solids, and mucus in relatively large quantities. This problem, however, is handled by the Costar® filtering step. Beyond this, it has been one author's (T.T.) experience that horse urine can contain quantities of fluorescent materials that vary markedly from horse to horse. This author has abandoned previous attempts to quantitate drugs in horse using fluorometry. However, the ability of PCFIA technology to bind the drug specifically in the immunoassay portion of the test and to wash the reaction system prior to testing for fluorescence increases the probability of success in this testing method. In addition, all that is required of a screening test is a high probability that a test is positive, and quantitation is not a concern.

Based on the data reported in this paper the PCFIA technology is more than sufficiently sensitive to detect the administration of fentanyl and its congeners to horses at the doses that are used to affect performance. As shown in Fig. 3 the assay can detect concentrations of fentanyl of less than 0.5 ng/ml. This is about the level of sensitivity afforded by the tritiated fentanyl radioimmunoassay assay (Michiels, et al., 1977), which has been sufficiently sensitive to control the use of fentanyl in racing horses. This tritiated fentanyl assay, however is unable to detect the use of carfentanil or sufentanil in racing horses.

In contrast, in the test reported here the specificity of the antibody is wide enough to react with other members of the fentanyl family. The antibody cross reacted with Sufentanil, Carfentanil, and the designer analogs, 4-methylfentanyl and 3-methylfentanyl. Given the level of cross reactivity presented in Table I, we elected to test whether or not this assay would detect each of these drugs after administration to horses.

As shown in Table II, this assay readily detected administration of fentanyl and sufentanil to horses. Administration of fentanyl at three different doses resulted in dramatic inhibition of the levels of fluorescence detected in the urine samples, indicating clear positives for fentanyl in these samples. Similarly, administration of Sufentanil at two different doses also resulted in easily detectable inhibition of fluorescence in urine samples from these horses. (Table II, Samples # 6a, 8a) Clearly, therefore, both fentanyl and sufentanil are readily detectable in post race urines by means of this test.

Among the fentanyls carfentanil is the most difficult drug to test for. It is the most potent of the fentanyls and cross reacts poorly with most antibodies raised to fentanyl. For this reason it is particularly important to have available a methodology that can detect carfentanil administration.

As shown in Table II this test also detected administration of carfentanil to horses. The smallest inhibition observed in a carfentanil treated horse was observed in the horse sampled in the first hour post dosing. At this time the levels of carfentanil in the urine would not have peaked and what carfentanil had entered the urine would be diluted by the drug free urine already present. Supporting

these observations, all the other carfentanil samples tested showed greater inhibition of fluorescence and were readily detectable as inhibited samples. The data suggest that this test is capable of detecting administration of carfentanil to horses at doses that are likely to be pharmacologically effective in a racing horse.

More recently several methylated analogs of fentanyl have become available on the illicit market, the so called designer fentanyls. These fentanyls appear to have the same pharmacological actions as fentanyl, and as shown in Fig 2, have about the same pharmacological potency as fentanyl. It was therefore of interest for us to evaluate the ability of this test to detect the administration of these analogs of fentanyl to horses.

As shown in Table III, use of the PCFIA readily detected the use of 3-methylfentanyl in horses. Fluorescence in control samples averaged about 27,453 units, and broadly similar fluorescence was observed in samples from control horses. All treated horses showed more than 60% inhibition of fluorescence, easily distinguishable from normal binding of the fluorescent material and therefore strongly suggesting a fentanyl positive.

Broadly similar results were obtained with α-methylfentanyl, although in this case the background fluorescence had to be corrected for inhibitors of fluorescence present in these urine samples. As shown in Table IV, the fluorescence readings obtained in control samples averaged about 27,458 in six individual determinations but the fluorescence levels were substantially less in track and control samples. When the baseline for reading the test in this case was taken as the mean of the control values from horse urine (23,499)

the test detected a methylfentanyl administration with good accuracy. However, the control samples from Remake still yielded a spuriously high inhibition fluorescence and thus a false positive.

The data on Remake (Table IV) point to the weakest aspect of this test, which is the possibility of natural fluors or quenching substances in horse urine affecting the results of the test. Natural fluors in horse urine could give rise to spuriously high levels of fluorescence in post-race urine samples, and thus mask the presence of fentanyls. On the other hand, quenching substances in horse urine could give rise to spuriously low levels of fluorescence, and thus to false positives. This second possibility, as seen in Table IV is less troubling, since all positives obtained by immunoassay are presumptive positives, until confirmed or otherwise by more specific testing methods. However, background fluorescence in horse urine may vary with season and diet, and for this reason the interpretation of PCFIA tests on racing samples will always depend on the observer's experience as to what is "normal" in that particular population. However, experience with the range of values experienced in normal horses is an absolute requirement for the application of any analytical methodology in racing horses.

REFERENCES

Combie, J., Shults, T., Tobin, T. (1979) The pharmacokinetics and behavioral effects of fentanyl and other narcotic analgeics in the horse. Proceedings. 3rd Intn'l. Symp. Eq. Med. Cont., Lexington. Pp. 311-321.

Jaffe, J., Martin W. (1985) Opioid analgesics and antagonists. In Gilman, A., Goodman, L., Rall, T. et al. (ed), The pharmacological basis of therapeutics. 7th ed, MacMillan, New York. Pp. 491-531.

Janssen, P.A.J. (1984) The development of new synthetic narcotics. In Estafanous, F.G. (ed) Opioids in Anesthesia, Butterworth, Boston. Pp. 37-44.

Jolley, M.E. (1983) Principles of particle concentration fluorescence immunoassay. Pandex Research Report #1, July. Pp. 1-2.

Jolley, M.E., Wang, C-H, Ekenberg, S.J., Zuelke, M.S., Kelso, D.M. (1984) Particle concentration fluorescence immunoassay (PCFIA): A new, rapid immunoassay technique with high sensitivity. J. Immunol. Meth. 67, 21-35.

Martin, W. (1984) Pharmacology of opioids. Pharmacol. Rev. 35, 283-323.

Michiels, M., Hendriks, R., Heykants, J. (1977) A sensitive radioimmunoassay for fentanyl. Europ. J. Pharmacol. 12, 153-158.

Moore, J.M., Allen, A.C., Cooper, D.A., Carr, S.M. (1986) Determination of fentanyl and related compounds by capillary gas chromatography with electron capture detection. Anal. Chem. 58, 1656-1660.

Tobin, T. (1981) Drugs and the performance horse, Charles C. Thomas, Publisher, Springfield, IL. Pp. 363-397.

Tobin, T., Combie, J., Miller, J.R., Crisman, M.W., Blake, J.W. (1979) 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. Ir. Vet. J., 33, 169-176.

Wie, S.I., Hammock, B.D. (1982) The use of enzyme-linked immunosorbent Assays (ELISA) for the determination of Triton X nonionic detergents. Analytical Biochemistry, 125, 168-176.