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Some reflections on positive results from medication control tests in the USA

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Summary

This article describes 3 of the drugs responsible for positive tests in American racing in recent years: fentanyl, apomorphine and reserpine. Experimental work is described in which the effect of administration was measured objectively against step counting; other aspects of locomotor stimulation and clinical responses are discussed. The supposed tonic effects of "pangamic acid" are considered and attention is drawn to the view of the US Food and Drug Administration that the substance does not exist.

Introduction

THE concept of medicating horses to improve their performance is probably as old as horsemanship itself. In ancient Rome honeyed water, called hydromel, was administered to chariot horses. The Roman chariot racing authorities took a serious view and crucifixion was, reputedly, the penalty for pre-race use. Whether or not this administration affected the performance of the chariot horses is not clear but nowadays, in most racing jurisdictions, it would not be considered a "doping" practice. Thus, what constitutes improper medication and appropriate punishment varies from culture to culture, and can sometimes vary even within the same culture (Tobin 1981).

A perspective of the problem of "positives" in the USA requires some background information about medication and racing. For the last 10 years or so, controlled medication programmes have been promulgated in most racing jurisdictions. These permit the use of certain drugs, most commonly phenylbutazone and frusemide, under certain restrictions. Some states have even more liberal programmes and may permit the use of other drugs. More recently, however, the controlled medication programmes have come under strong attack by various pressure groups in the USA and the future of these rules is in doubt. At present there is a bill before Congress, causing substantial concern in the US racing industry, which would make it a felony to medicate a racing horse.

In this context, finding a test positive for phenylbutazone or frusemide in the states which ban these drugs is not a remarkable event. However, all racing jurisdictions in the USA ban the use of stimulants, depressants, local anaesthetics and tranquillisers.

Three of the most interesting drugs responsible for positive tests in American racing in recent years are discussed here.

Fentanyl

Fentanyl, or sublimase, is a synthetic narcotic analgesic of the morphine family (Tobin, Combie and Dougherty 1979a). Like morphine, fentanyl is a stimulant in horses, but is about 80 times more potent. This potency confers a particular advantage because it is, therefore, 80 times more difficult to detect, and for several years in the USA, the users of fentanyl were ahead of the analysts.

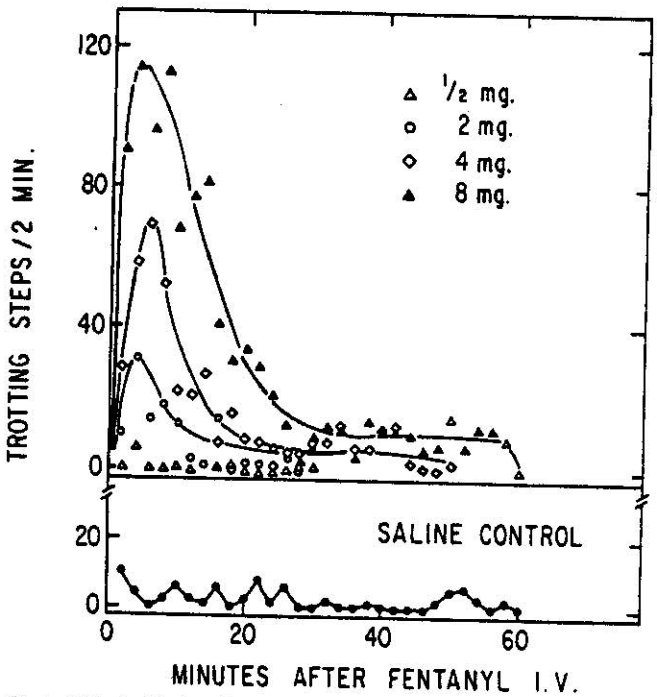

Fig 1. Effect of fentanyl, at various dose levels, and saline (control) on locomotor activity in a single horse

In our initial experimental work small doses (approximately 0.5 mg/horse) were administered with few consistent effects. Larger doses (more than 4 mg/horse) showed a clearcut stimulant response. A simple and effective method (step counting) of measuring stimulant responses to fentanyl and similar agents has been devised (Tobin *et al* 1979a).

Step counting

The horse is placed in a loose box and, after a period to allow it to settle down, the number of steps that it makes with its left front foot is recorded by an observer equipped with a stop watch, an event counter and log book (Fig 1) (Tobin *et al* 1979a).

No response is obtained employing the relatively small doses of fentanyl which are reported as being used on the racehorse. If, however, the dose is increased 10-fold, a very dramatic response is obtained. The peak response is obtained when 8 mg of drug is given rapidly intravenously (iv) and the horse moves as fast as is possible in the loose box.

This effect lasts for about 30 mins. It is characteristic of a horse's response to narcotic analgesics that an increased dose causes incoordination and recumbency. There is, therefore, quite a narrow dose and time range for the optimal stimulant effect.

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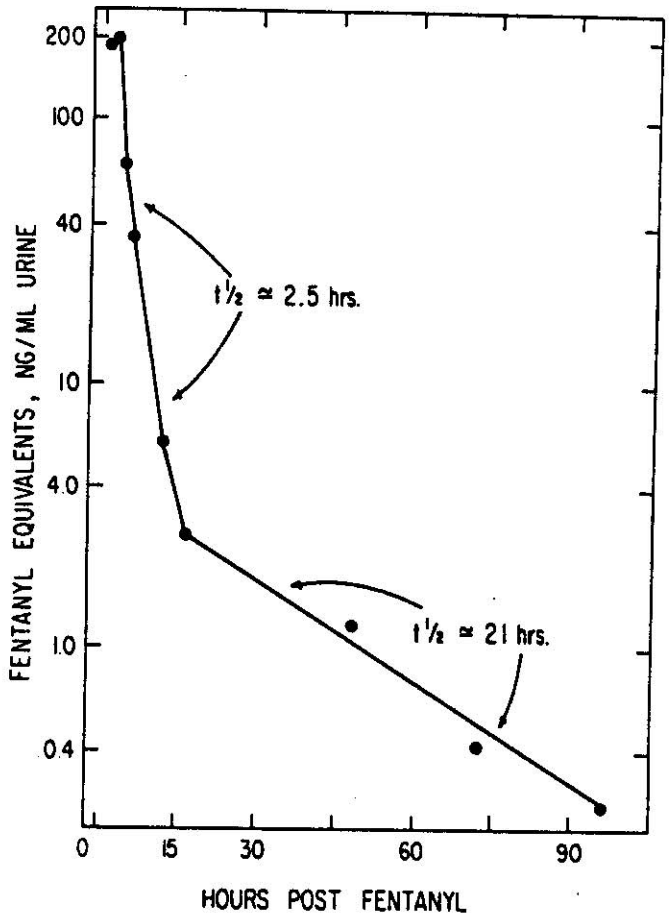

Fig 2. Urine levels of fentanyl and, or, its metabolites after 0.02 mg fentanyl/kg body weight intravenously in a single horse. Apparent urinary half-lives were approximately 2.5 and 21 h

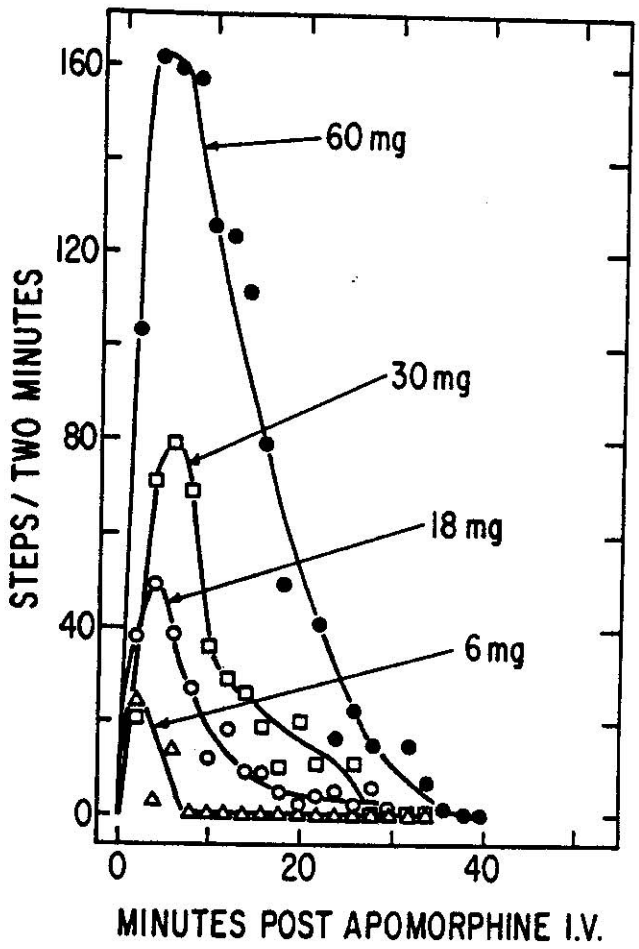

Fig 3. Effect of (iv) apomorphine at various dose levels on spontaneous locomotor activity in a horse.

Use and detection

Fentanyl, at the optimal dose and time, is quite a potent locomotor stimulant in the horse. It is not clear whether or not the small dose (about 0.25 mg/horse) used on the racecourse and administered intramuscularly (im) is pharmacologically effective. However, it was this dosage which was reputedly widely used on tracks in the USA in the late 1970s. According to the popular press, many racehorses were being given fentanyl and there was substantial pressure on racing chemists to develop a method for its detection. Such a method has been developed using radioimmunoassay (Michiels, Hendricks and Heykants 1978), and fentanyl can be detected in a urine sample for up to 4 days after administration (Fig 2). However, radioimmunoassay does not specifically identify a chemical and a reliable confirmatory method was developed by Mr John MacDonald of the Illinois Racing Board Laboratory (personal communication). Positive results for fentanyl were soon reported and, at present, about 150 cases have occurred. Although a number of these cases are still before the courts, the use of fentanyl in American racing is essentially finished.

From a forensic viewpoint, the handling of these fentanyl positives was, in general, satisfactory. Mass spectrometry was used as a basis for the positive reports, which were confirmed by independent laboratories. Split samples were made available to the defendants for analysis by chemists of their choice. All of these independent analyses showed the presence of a fentanyl metabolite in the suspect urine samples. Although some trainers chose to challenge their racing authority in the courts, none did so on chemical grounds. One trainer in Massachusetts successfully defended himself on the grounds that the test had been performed, contrary to the regulations, by somebody other than the official chemist. However, most of the positive identifications have been upheld in the courts and suspensions have been imposed on the horsemen involved.

Apomorphine

Apomorphine is derived from morphine by treating it with strong acid; this transformation completely changes the pharmacological activity of the drug. Apomorphine has no narcotic analgesic properties but acts as a dopaminergic stimulant. One of the characteristics of these agents is that they powerfully stimulate running in most species (Tobin, Combie and Shults 1979b). This effect is illustrated in Fig 3. A horse was injected intravenously with the indicated doses of apomorphine and its trotting activity measured by the step counting method. There was a very rapid increase in trotting after injection of the drug, and trotting activity was much faster after apomorphine than after narcotic drugs. Also, the rate of decline of the trotting response to apomorphine appears to be faster than that observed after fentanyl.

However, it was found that a high dose of apomorphine did not produce incoordination and ataxia (Tobin *et al* 1979b).

Comparison of fentanyl and apomorphine

While apomorphine produces a locomotor stimulation which closely resembles that produced by narcotic analgesics, there were a number of characteristic differences between the actions of these drugs.

Apomorphine-treated horses remained well coordinated at all doses tested. On high doses they achieved rates of 160 steps/2 min period, at which point they were moving as rapidly as was possible in the stall. In contrast, as the dose of fentanyl was increased and the horses surpassed rates of about 100 steps/2 min period, they showed impairment in co-ordination and occasionally bumped into the walls of the stall. If the dose of fentanyl was further increased, the animals became uncoordinated and fell.

Unlike the dreamy, dissociated appearance of animals on high doses of narcotics, horses on apomorphine appeared apprehensive and uncomfortable with the presence of the observer. While horses on fentanyl circled, horses on apomorphine always kept at a distance from the observer. Locomotor activity, after administration of apomorphine, usually took place along the wall of the box distant from the observer, with the horse pacing back and forth repeatedly. If the animal was extremely excited and apprehensive, it lunged up toward the ceiling as it reached each corner, as though seeking to escape. At this point it would abruptly change direction, pace back along the wall to the other distant corner of the box and repeat the process. All these manoeuvres were carried out at speed, as the pacing rate of up to 160 steps/2-min suggests, and the horses were well coordinated throughout. If the dose of apomorphine was further increased, the horses tended to injure themselves on the walls.

In addition to this remarkable increase in locomotor activity, horses dosed with apomorphine emitted a snort or snore during the period of drug action. This sound was so characteristic it was called the apomorphine snort, and it has also been reported by Mackay (1961). Although horses dosed with narcotic analgesics showed many behaviour patterns suggestive of dopaminergic stimulation, they never emitted this typical apomorphine snort.

It is not clear how the locomotor stimulating effects of apomorphine and fentanyl relate to racing performance. Most studies on the performance effects of drugs have been carried out with other agents (Fujii *et al* 1970, 1974, 1977; Sanford 1971, 1973; Steward 1972) and few performance experiments with narcotic analgesics have been reported. It would be incorrect to assume that because these drugs stimulate locomotor activity they must, necessarily, improve racing performance. Nevertheless, these experiments do suggest that the narcotic analgesics and dopaminergic stimulants, such as apomorphine and pemoline (Tobin 1981), merit more careful testing in future performance experiments than they have received in the past.

Reserpine

Reserpine is the principal alkaloid of a climbing shrub of the Apocynaceae family called *Rauwolfia serpentina*, which is indigenous to India and neighbouring 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. These uses went unnoticed by modern western medicine until Sen and Bose (1931) described its use for treatment of psychoses and hypertension in the Indian medical literature. This report attracted the attention of western investigators to rauwolfia, and pure reserpine was isolated from the crude plant material in 1952. When introduced in this form, reserpine was the first of the modern major tranquillisers and, in the 1950s and early 1960s, was widely used in human medicine for the treatment of psychoses and hypertension. It soon became apparent, however, that its use was associated with severe side effects, such as Parkinsonism, depression, suicide and seizures, and reserpine is currently used only in patients resistant to other forms of therapy. However, reserpine is still widely used in horses as a tranquilliser because of its efficacy and its long duration of action after a single dose (Tobin 1979).

Reserpine is a remarkably potent drug and very small doses (less than 5 mg) in the horse can produce considerable biochemical and behavioural 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, ie, a drug which produces a biochemical change which persists for days after the drug has been eliminated from the body. This concept is now regarded as incorrect and it has been suggested that reserpine is 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.

A dose of 5 mg of reserpine produces a clinical effect for about 2 days. During this period the individual shows signs of penile relaxation, diarrhoea, sweating and drooping of the eyelids. An apparent recovery occurs 72 h after dosing. However, an apparatus has been developed (Shults, Combie, Dougherty and Tobin 1979; Tobin 1981) whereby a horse breaks a light beam for reward and this has demonstrated that the tranquillising effect of a dose of reserpine lasts for up to 10 days after a single dose although the readily observable clinical signs disappear within 3 days. Reserpine is, therefore, a very subtle tranquilliser, with an effect that can only be detected by relatively sophisticated apparatus, or a horseman quite familiar with the individual horse (Shults *et al* 1979).

Reserpine is widely used, legitimately, in equine medicine in the USA but it has been illegally employed to depress race favourites and show horses have been treated with reserpine before an event to calm them. The authorities concerned had, therefore, a particular need for a test for the drug.

Detection

In 1978, a high performance thin layer test for reserpine was developed (Ray, Sams and Huffman 1978) and used to detect the first reserpine positives in the USA. Although this test was challenged by a number of horsemen, its use by the American Horse Shows Association was supported by the New York State Court system. Its introduction, and the subsequent development of independent confirmatory tests, has had the effect of eliminating the use of reserpine in show horses.

Pangamic acid

Pangamic acid was developed by Ernest Krebs, Sr and Jr who, in 1943, applied for a patent for a material isolated from apricot seeds, which they named pangamic acid, and for which they used the trade name Vitamin B_{15}. Since then, pangamic acid has been widely promoted both as a food supplement for horses and for the relief of numerous human ailments, including heart disease, diabetes, drug addiction, jaundice, neuralgia and neuritis. It has had a considerable following as a health food and is widely accepted as a necessary food factor with important physiological actions. Pangamic acid also has a considerable reputation in athletic circles. It has been fed to the horses of the American Equestrian Team and has been used by Muhammed Ali. Pangamic acid is reputed to produce its tonic and vitamin effects by increasing the supply of oxygen in the

blood and its uptake in tissues, although quite how it increases the supply of oxygen in the first place is not clear. Many athletes consume quantities of B_{15} although there is no evidence that it improves their performance. The dosage recommended has varied from 50 mg/day in Russia to 150 mg/day in the USA.

The pangamic acid described may have been a sodium or potassium salt of d-glucunodimethylaminoacetic acid. However, this label frequently describes a product which is one part gluconate, one part glycine or dimethylglycine and, often, one part di-isopropylamine dichloroacetate. Because of this chemical variety in the materials sold as pangamic acid some authorities, including the US Food and Drug Administration, hold that the substance does not exist. There is, therefore, no standard chemical identity for products sold as 'pangamic acid'. However, despite the apparent non-existence of this substance, it has recently been reported as being detected in horse urine in at least one racing jurisdiction.

Acknowledgements

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TAPE REVIEW

Abnormal oestrous cycles in the mare by W. E. Allen. Available from the Unit for Veterinary Continuing Education, Royal Veterinary College, London NW1 0TJ. Price £16.10.

THIS tape cassette recording is produced by the Unit for Veterinary Continuing Education at the Royal Veterinary College and should be used in combination with an accompanying booklet which contains useful diagrams and glossary of terms. The author points out clearly that the programme is designed for veterinary students and practitioners who occasionally have to carry out gynaecological examinations.

Part 1 of the recording describes the normal oestrous cycle, the environmental and physiological factors which may result in deviations from normality and methods of treatment of abnormalities. The use of synthetic progestogens receives scant attention and the practice of progesterone assays is discussed without any indication of the normal levels; unfortunate, perhaps, when commercial assays are available to all practitioners.

Part 2 of the programme describes the techniques of rectal and vaginal examination and the interpretation of the observations.

The producers of this programme are to be congratulated on their clear and useful production which will be helpful to students and inexperienced practitioners who prefer the spoken to the written word.

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