

Trace element, toxin and drug elimination in hair with particular reference to the horse

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1. Introduction

From a veterinary perspective, hair must be regarded as a much neglected tissue. This is unfortunate, but not surprising, since hair (as opposed to the skin from which it grows) is not associated with major pathological disease and the physiology and biochemistry of hair in animals have not been extensively studied. Whilst hair is a living tissue, it is metabolically relatively inert and, once formed, does not undergo further biogenic turnover. Its formation and growth is regulated by physiological processes, enabling responses, for example to environmental influences. Whilst early anatomical opinion viewed the skin and hair simply as a passive barrier to fluid loss and mechanical injury, it is now recognised that hair performs a range of integrated functions (Table 1). For example, it is integral to body temperature regulation and provides a protective barrier against the animal's environment (Stenn and Paus, 2001; Tregear, 1965). Thus, hair density is greater over regions of the skin exposed to direct sunlight (Pilliner and Davies, 1996). Coat colour impacts on thermal regulation, light coloured coats being more effective than darker colours in hot environments (Lyne and Short, 1965; Scott, 1988). In addition, glossiness of coat hair, as found in tropical equine breeds, assists the reflection of solar radiation (Hayman and Nay, 1961; Holmes, 1970).

In equine skin several hair types are recognised: temporary hair makes up the majority of the coat; hair of the mane, tail and eyelashes is permanent and tactile hairs are located in or near the muzzle, ears and eyes. The anatomical location of permanent hairs provides protection in several ways. The mane assists the shedding of rainwater and insulates the head and major

blood vessels to the brain (Pilliner and Davies, 1996), whilst the eyelashes protect against corneal impact injury.

2. Hair structure, composition and growth

2.1. Hair shaft structure

The hair shaft derives from hair follicle growth. Structurally, there are three distinct components: a central medulla, a protective outer cuticle and the cortex located intermediately (Harkey, 1993). The 'tiled' structure of the outer cuticle is due to overlapping cells, which fix the hair shaft to the follicle by interlocking with cells of the inner root sheath.

The greatest bulk of the hair shaft is made up of the cortex containing longitudinally oriented, spindle shaped keratinocytes. The cells are composed of microfibrils, or keratin bundles, which comprise approximately 85% of the cortex (Cone and Joseph, 1996). The keratin protein fibres cross-link to provide the hair with its mechanical strength, and the structural proteins are inter-spaced with air gaps termed fusi. The cortex also contains melanin (eumelanin providing black/brown pigmentation and pheomelanin red/yellow pigmentation) granules. Melanin is even more resistant than keratin to enzyme and microbial attack. The medulla comprises randomly orientated and loosely packed rectangular cells, rich in the structural protein trichohyalin, which is less resistant than keratin. When dehydrated, medulla cells shrink to leave empty vacuoles along the central axis of the hair shaft (Chatt and Katz, 1989). The numbers of medullary cells and hence medullary area increase with increasing hair fibre diameter. Thus, the fine hairs of the equine coat are made up mainly of cuticle and cortex cells, whilst mane and tail

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Table 1
Functions of hair (from Stenn and Paus, 2001)

Functions of hair
Insulation against heat loss and gain
Sensory assessment of the environment
Protection against trauma, insect penetration and electromagnetic radiation
Decoration, social communication and camouflage
Mechanism of outward transport of social environmental signals (sebum and pheromones)

hairs contain a greater number and a greater proportion of medulla cells (Harkey, 1993; Talukdar et al., 1972).

Chemical composition of the shaft. Hair may be described as a crystalline, cross-linked and orientated polymeric protein structure. As well as protein, the hair shaft contains melanins, water, lipids and inorganic minerals. Approximate percentage proportions in human hair are, respectively, 80–85 (protein), 0.3–1.5 (melanins), <15 (water), 1–9 (lipids) and 0.25–0.95 (minerals).

The principal hair proteins are three structurally related keratins, the low-sulphur, high-sulphur and high-tyrosine, high-glycine keratins. The sulphur content derives from sulphur-containing amino acids, principally cysteine. Hair lipids include free fatty acids and triglycerides. The melanins are polymers formed in melanocytes by the oxidation of tyrosine. The melanin content of human hair has been found to vary between individuals and between races (Borges et al., 2001). The keratinised region of the hair shaft, extending beyond the skin epidermis as visible hair, is dehydrated. Hair water content derives from sweat and atmospheric moisture; and varies directly with environmental humidity (Robbins, 1979). Several heavy metals, such as lead, cadmium and mercury, and trace elements are present in hair. The concentrations of these different constituents vary with factors such as diet, disease, genetics and weathering.

Hair follicle structure. The hair follicle that forms the hair shaft contains vascular, muscular and glandular components (Chatt and Katz, 1989). Follicles vary in structure with differing anatomical locations and are thus capable of generating hair shafts of differing size, shape, curl and colour (Stenn and Paus, 2001). Some species, including dogs and cats, have compound hair follicles which produce primary and secondary hairs, whereas the horse has simple follicles that form exclusively single hairs (Lloyd, 1993; Talukdar et al., 1972).

Linked to each simple follicle are apocrine sweat and sebaceous glands and an arrector pili muscle, contraction of which erects the hair shaft, thus regulating ventilation and heat loss. Erection is also associated with the sympathoadrenal fight and flight response to perceived danger. Sebum is a lipid-based waxy substance, formed by the sebaceous glands, which coats the hair and skin to repel water and provide a physical barrier. Sebum also inhibits the growth of microorganisms

(Lewis, 1995) and retards the penetration of toxic substances (Vale and Wagoner, 1997). The apocrine glands excrete an oil that, like sebum, coats the hair.

The follicle also contains a dermal papilla, an inner and outer root sheath, and a bulge region. The papilla regulates follicular development by providing a permissive signal for hair growth. The hair bulb comprises proliferative epithelial cells. These produce the hair matrix and the inner and outer root sheaths (Lloyd, 1993). The hair follicle is an organ, containing several enzyme systems, that determine the biochemical composition of hair (Jarrett, 1977; Potsch et al., 1997).

2.2. Hair growth

The rate of growth of human scalp hair is 0.7–1.5 cm/month (Harkey, 1993). The hair shaft grows through the formation of matrix cells within the bulb. Cell differentiation enables formation of the layers of the shaft and the surrounding root sheaths. As the shaft reaches the follicular bulge area, keratinisation leading to hardening occurs by a process of protein cross-linking through highly stable disulphide bridges between adjacent cysteine molecules. The relative resistance of hair to degradation is due largely to the cysteine cross-links. The hair shaft then extrudes from the skin and the rate of hair growth is determined by the rate of cell proliferation (Blume et al., 1991).

The growth cycle of hair follicles includes a long period of active hair growth (anagen), a short transitional period of slow growth (catagen) and a rest period of no growth (telogen), after which shedding of the hair shaft (exogen) occurs (Harkey, 1993; Lloyd, 1993; Stenn and Paus, 2001). In anagen the follicle actively forms new hair shaft. In catagen new growth ceases and shrinking of the follicle occurs until, in telogen, an inactive club hair is formed (Randall and Ebling, 1991). As telogen ends, a further anagen phase, involving regeneration of hair matrix from stem cells in the permanent part of the follicle, occurs under the regulatory control of the dermal papilla (Gailbraith, 1998). This leads to formation of a new hair shaft and its growth causes shedding of the previous club hair. At any one time, in human adults approximately 85% of scalp hair is in the growing phase.

Although extensive data are not available, it is clear that the length of the hair growth cycle and the duration

of each phase differ between individuals, species and anatomical sites. The growth cycle provides the means by which animals alter their pelage to meet the needs of regeneration and seasonal fluctuations in climate (Randall and Ebling, 1991).

2.3. Non-dietary factors affecting hair growth rate in horses

There is continual growth of the permanent hairs of the equine mane and tail. Investigations in a small numbers of horses over short intervals indicated a relatively constant rate of growth of mane hairs (Popot et al., 2000; Whitem et al., 1998). Studies in our laboratory involving 29 horses of various breeds have shown that both mane and tail hair growth is relatively constant over a 12-month period (Fig. 1). Month by month comparisons indicated some variation in rates of hair growth in both mane and tail, but overall there was no clear correlation between these fluctuations and either climatic or seasonal factors. Growth rate differed slightly within three regions of the mane (Fig. 1), being slowest near the withers, highest near the poll and intermediate near the crest. In both mane and tail, growth rates were faster in native pony breeds than in thoroughbreds and intermediate in cross-breeds (Tracey et al., 2002). This study indicated no demonstrable effect of age or gender on rate of hair growth rate in the tail and mane.

Seasonal hair growth and shedding of the pelage is well recognised in horses and domestic pets, as well as wild animals. Thus, in cattle and in cats hair growth is absent or minimal in winter (Baker, 1974; Dowling and Nay, 1960; Ryder, 1976). In sheep, wool growth rate peaks in summer and early autumn (Coop, 1953), whilst

in humans slightly faster hair growth occurs in late summer and early autumn (Courtois et al., 1996; Randall and Ebling, 1991).

In spring, late summer and early autumn changes in photoperiod, through the eyes and by several endocrine pathways, affect hair growth. In the horse the onset of pelage and the rate of shedding is increased by artificially extended photoperiods in both fillies (Wesson and Ginther, 1982) and mares (Kooistra and Ginther, 1975; Oxender et al., 1977). Pelage responses to photoperiod change in pony colts were delayed, lagging behind day length changes by 5–8 weeks (Fuller et al., 2001). Seasonally, the fastest growth occurs in the autumn (Popot et al., 2000). The influence of photoperiod on the permanent hairs of the tail and mane has not been determined, but data from our laboratory suggests a tendency for the growth of mane and tail hair to be greatest in autumn.

Regulation of secretion of the pineal hormone melatonin in relation to mammalian pelage is mediated through light receptors in the eye, which signal changes in daylight length to the pineal gland. Melatonin synthesis and release increase as daylight length decreases (Bergfelt, 2000).

The effect of androgenic steroids on hair growth in horses is unknown, but red deer stags produce long mane hairs in the breeding season under the influence of androgens (Thornton et al., 2001). Circulating androgen levels in humans may influence hair growth, although this is not clearly established (Messenger, 1993; Randall and Ebling, 1991).

In male horses, seasonal increases in blood prolactin levels correlate with shedding of the winter coat (Argo et al., 2001), and recombinant porcine prolactin administration to seasonally anoestrous mares led to

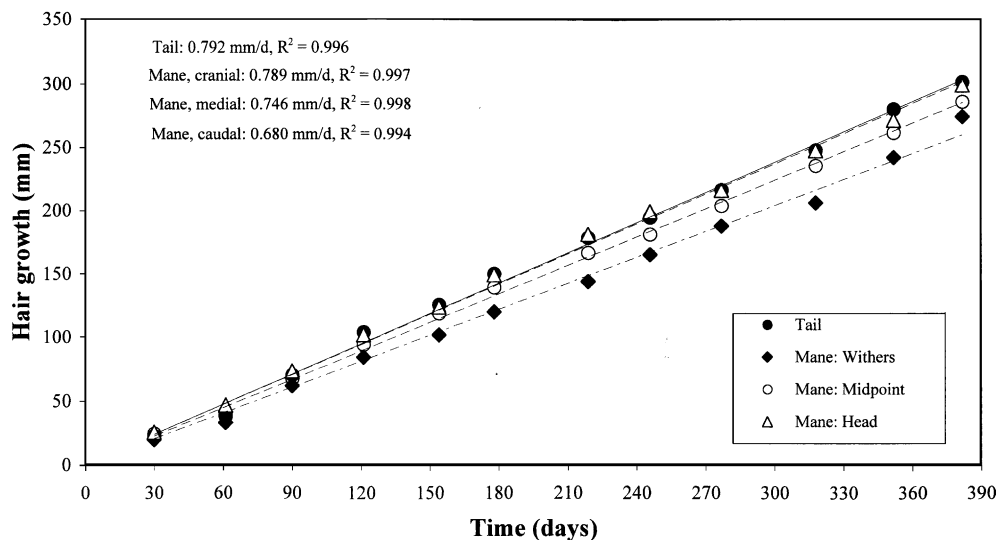


Fig. 1. Mean cumulative mane and tail hair growth for a group of continuously grazed native ponies ($n = 5$). Regions of the mane and tail were shaved to the skin and subsequent re-growth was measured monthly over the subsequent 12 months.

pelage shedding within 14 days (Thompson et al., 1997). The thyroid gland also affects hair growth; enhanced growth occurs in response to increased circulating thyroxine levels in human subjects (Parker, 1981) and dogs (Gunaratnam, 1986). On the other hand, hypothyroidism is commonly associated with diffuse alopecia (Ebling, 1981). Although data in horses are limited, coarser coat hair growth has been reported in thyroidectomised mares (Lowe et al., 1987).

Whilst the effect of daylight length on melatonin and prolactin secretion, and hence on pelage growth, is well known, there is no information on the effects of these hormones on equine mane and tail hair growth rates. Likewise, there are few data on the influence of climatic factors, such as temperature, intensity of solar radiation and relative humidity. However, young standard-bred horses when cold-housed produced up to twice as much coat hair as warm-housed horses of the same age and breed (Cymbaluk, 1990).

3. History of hair analysis

Casper (1857–1858) detected arsenic by hair analysis 11 years post-mortem in a suspected murder victim. There seems to have been no further interest in hair analysis for more than 80 years thereafter, until Flesch (1945) suggested that hair might be regarded both as a metabolic end product and excretory organ, the trace element content of which reflected the medium from which it was formed. Subsequently, the heavy metal content of hair was described by Goldblum et al. (1953), and the same group (Goldblum et al., 1954) provided the first report of detection of an organic drug, phenobarbitone, in guinea pig hair. Both Forshufvud et al. (1961) and Smith et al. (1962) undertook retrospective hair analysis to investigate the possibility that the Emperor Napoleon had been poisoned with arsenic. Their analysis revealed repeated exposure to arsenic. However, no firm conclusion regarding the arsenic source could be drawn, since pigments containing arsenic were used in wallpaper manufacture in the early years of the 19th century. Metabolites of cocaine and its parent compound were detected retrospectively in Peruvian mummy hair more than 500 years old (Springfield et al., 1993).

Analysis of human hair to monitor nutritional trace element content and to identify and track exposure to heavy metals was used throughout the 1960s and 1970s. An example is suspected mercury poisoning in Iraq, resulting from consumption of bread prepared from grain contaminated with mercury-based fungicides (Giovannoli-Jakubczak and Berg, 1974). Likewise, hair analysis was used to monitor occupational and lifestyle exposures to such heavy metal toxins as mercury in dental technicians (Leniham et al., 1973) and lead de-

riving from traffic exhaust emissions in school children (Hammer et al., 1971).

Hair analysis as a means of detecting the abuse of controlled drugs and establishing in individual human subjects a history of drug use was introduced by Baumgartner et al. (1979). This group detected opioid drugs in hair samples from addicts by a radioimmuno-logical method. Shortly thereafter, hair analysis was extended to tracking other drugs of abuse, including barbiturates (Smith and Pomposini, 1981), phencyclidine (Baumgartner et al., 1981) and cocaine (Valente et al., 1981). Since then there have been major advances in analytical methodology, sensitivity and validation to enable detection of a wide range of drugs of abuse and therapeutic agents in human hair (Gaillard and Pepin, 1999; Nakahara, 1999; Tagliaro et al., 1997).

Hair is not generally regarded as a major excretory organ for endogenous or exogenous (including drugs and toxins) compounds. Quantitatively, amounts eliminated in hair, expressed as a percentage of administered dose, are inevitably small. However, compared to most body tissues, hair (itself very resistant to environmental forces) provides a very stable medium, in which trace elements, minerals, drugs, toxins and their metabolites can be protected and detected over prolonged periods. Hair analysis can thus provide a historical record of drug (or other chemical) exposure, even though some losses may occur due to chemical change or leaching out. As well as detecting drugs or chemicals retrospectively months or years after systemic exposure, hair root analysis may indicate acute exposure. Gygi et al. (1995) detected codeine in root hair one hour after administration. The attraction of hair as a matrix for analysis lies also in the fact that it is easily collected, transported and stored and methods for the extraction and chemical analysis of a wide range of compounds at low concentrations have been established and validated. Potential routes for the incorporation of drugs and other substances, including trace minerals, into hair are illustrated in Fig. 2. Entry may be gained through capillaries supplying nutrients to the follicles, in sebum, oil or in sweat. However, whilst the latter secretion is available to the horse, sweating does not occur in the cat or dog.

4. Assessment of nutritional status by hair analysis

The assessment of nutritional status, including essential elements and trace minerals, by hair analysis has been used for many years and within the last 20 years increasing use has been made of spectroscopic methods to facilitate multi-element analysis. Current analytical techniques provide reliable, rapid and relatively inexpensive diagnostic methods (Chyla and Zyrnicki, 2000). Hair contains high concentrations of many trace elements, and has been used to monitor nutritional status

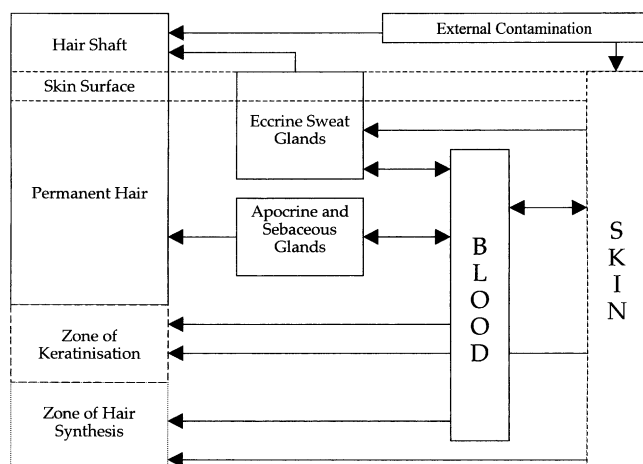


Fig. 2. Proposed multi-compartment model for the incorporation of drugs and other substances into hair (adapted from Henderson, 1993).

over extended time periods. Compared to other matrices, such as plasma and urine, hair analysis circumvents the transient fluctuations arising from recent or variable dietary intake.

Attempts have been made to use hair analysis as an indicator of the whole body status of minerals, such as calcium and phosphorus (Sippel et al., 1964; Wysocki and Klett, 1971) and trace metals such as copper, molybdenum, zinc, selenium and iron (Cape and Hintz, 1982; Wichert et al., 2002). However, there are still uncertainties as to whether hair content is well correlated with whole body levels and the validity of the approach in the horse remains to be confirmed (Hintz, 2000).

5. Hair analysis to monitor environmental toxin and heavy metal exposure

Hair analysis has been used to track the history of human exposure to toxic heavy metals, such as cadmium, mercury and arsenic (Chatt and Katz, 1989). There has been less extensive use of hair analysis in animal toxicological studies. However, there are reports of environmental exposure of wildlife to heavy metals (Burger et al., 1994) and selenium (Clark et al., 1989; Edwards et al., 1989), and to selenosis in domesticated species (Mihajlovic, 1992). Lead levels in coat hair of animals grazing pasture near to a lead smelter were significantly increased (Levine et al., 1976). In horses in central Europe, environmental exposure to cadmium in relation to age, breed, gender and location has been investigated by hair analysis. Cadmium accumulated to a greater extent in geldings than mares (Anke et al., 1989). The exposure of horses, sheep and alpacas to several toxic heavy metals and other elements, including cadmium, lead, chromium, nickel and bromine, from vehicle emissions was investigated by hair analysis by

Ward and Savage (1994). Increased lead and cadmium concentrations were detected in equine hair and blood, with a significant correlation between blood and hair levels of lead. Toxic levels of selenium in forage were strongly correlated with selenium concentrations in coat, mane and tail hair. Hair selenium concentrations ranged from 0.3 to 7.1 mg/kg (Witte et al., 1993).

In humans, pesticides including 1,1-dichloro-2,2-bis (*p*-chlorophenyl)ethylene (DDE) and other polychlorinated biphenyls were detected in hair in concentrations of 0.5–4.9 pg/kg (Dauberschmidt and Wennig, 1998).

The application of hair analysis to the detection and monitoring of plant-induced toxicoses in horses would be a useful application. It has been estimated that approximately 500 horses die each year from hepatic disease caused by the ingestion of Common (or Tansy) Ragwort in the UK alone. The hepatotoxins causing primary hepatic failure are pyrrolizidine alkaloids (Lewis, 1995). It is predictable that these and other alkaloids will be deposited in equine hair.

6. Drug elimination in equine and canine hair

6.1. Potential uses of drug detection and quantification in hair

The elimination of drugs and their metabolites in hair is potentially of great value in relation to sports anti-doping control, as well as in pre-purchase vetting and residue monitoring in stock production. Illegal drug use in animals may arise in several ways. The growth-promoting properties of anabolic steroids, such as testosterone, nandrolone and stanozolol, and repartitioning agents, including clenbuterol, albuterol and brombuterol, can be used illegally to enhance muscular development in equine bloodstock and cattle breeding programmes. Thus, Appelgren et al. (1996) detected clenbuterol in calf hair. In addition, a wide range of performance altering drugs, including local anaesthetics, CNS depressants and stimulants and drugs acting on the cardiovascular system, may be misused both during training and prior to participation in competitive equine and canine sports. Furthermore, anti-inflammatory corticosteroids such as dexamethasone and non-steroidal anti-inflammatory drugs (NSAIDs), such as phenylbutazone, may be used abusively prior to competition or to mask lameness in horses before pre-purchase veterinary examination. A further potential application of hair analysis is in the detection of drugs used in therapy but which are not authorised for use in horses intended for human consumption.

Recent equine studies have demonstrated the potential of hair analysis to provide additional analytical evidence to that obtained from blood or urine analyses. In contrast to urine and blood analyses, however, hair

analysis can detect and quantify drugs weeks, months or even years after administration or intake. A further consideration is that racehorses may fail post-race tests for prohibited substances through ingestion of a number of contaminants naturally present in feedstuffs (Table 2). In human medicine hair analysis has been used to monitor compliance with long term therapeutic drug regimens, for example for anti-epileptic drugs and Dunnett et al. (2002a) have demonstrated a similar potential for phenobarbitone use in epileptic dogs.

6.2. Mechanisms and correlations of drug elimination in hair

The elimination of drugs, and indeed other substances, in hair is not necessarily a passive process. The supply, accumulation and subsequent persistence of drugs in the hair shaft are achieved through a range of poorly understood mechanisms, involving not only physico-chemical properties of drugs but also depending on the animal's anatomy and physiology. It is therefore of interest to explore these inter-relationships, so that the science of hair analysis may be taken beyond their detection and quantitation. Accordingly, in 1998 a research programme in our laboratory commenced with the aim of exploring possible correlations between drug concentration in mane and tail hair on the one hand and a range of factors such as administered dose, drug pharmacokinetics and the constituents of hair on the other. Our objectives have been to (a) establish and validate methods of extraction and chemical analysis of therapeutic and illicit drugs and their metabolites in equine mane and tail hair; (b) evaluate the potential of hair analysis for the retrospective detection of drug use and misuse in horses, including investigation of the relationship between concentration and hair growth rates; (c) establish relationships between administered dose and pharmacokinetic parameters for drugs in plasma (such as AUC and C_{max}) and the level of accumulation and persistence in hair; (d) determine the importance of hair-related factors such as melanin content and drug concentration achieved in hair, including tracking drug concentration–time relationships by segmental analysis;

and (e) determine the influence of drug-related factors such as lipid solubility, acid–base properties and binding to plasma protein and the level of accumulation.

Regarding objectives (a) and (b), we have established analytical methods for and detecting in equine hair many of the drugs listed in Table 3. Regarding objectives (c–e), to penetrate cell membranes the drug must have some degree of lipid solubility. Most drugs are either weak acids or weak bases and are partially ionised at physiological pHs. The unionised fraction is generally lipid soluble and will penetrate cell membranes readily. However, pH may differ intra- and extra-cellularly and weak acids are diffusion trapped in alkaline environ-

Table 3
Drugs detected to date in equine hair

Drug detected	Metabolite detected
Trimethoprim ^{a,d}	No
Sulphadiazine ^{a,d}	No
Sulphadimidine ^e	No
Metronidazole ^{a,d}	No
Procaine ⁱ	No
Enrofloxacin ^f	Ciprofloxacin and others
Etamiphylline ^c	Desethyletamiphylline and others
Pentoxyfilline ^c	Lysofylline and others*
Caffeine ^c	Theobromine and theophylline
Xylazine ^b	No
Methocarbamol ^b	No
Morphine ^h	No
Diazepam ^f	No
Clenbuterol ^f	No
Stanozolol ^g	No
Boldenone ^g	No
Salicylate ^e	No
Griseofulvin ^c	No
Omeprazole ^c	5-Hydroxyomperazole*
Carprofen ^c	No

^a Dunnett and Lees (2000).

^b Dunnett et al. (2002c).

^c Dunnett et al. (2002b).

^d Dunnett et al. (2003).

^e Dunnett and Lees (unpublished data).

^f Popot et al. (2000).

^g Popot et al. (2002).

^h Whitem et al. (1998).

ⁱ Dunnett and Lees (2002).

* Identity not confirmed.

Table 2
Prohibited substances present in feedstuffs

Chemical classification	Pharmacological classification	Examples
Alkaloid	Muscarinic receptor antagonists	Atropine and hysocine
Alkaloid	Narcotic-analgesics	Morphine
Alkaloid	Phosphodiesterase inhibitors	Caffeine, theophylline and theobromine
Alkaloid	Hepatotoxins	Pyrrolizidines
Organic acid	Anti-inflammatory-analgesic	Salicylate
Volatile oil	Respiratory stimulant	Camphor and menthol
	Miscellaneous	Borneol, bufotenine, dimethyl sulphoxide, hordenine, lupanine, oryzanol and sparteine

ments and vice versa for weakly basic drugs (Henderson–Hasselbalch mechanism). The isoelectric pH of hair is close to 6 (more acid than plasma) and this favours the accumulation of weakly basic drugs in matrix cells (Robbins, 1979). However, there is an additional factor that favours even greater accumulation of basic drugs; melanocytes have an intracellular pH in the range of 3–5 and this leads to diffusion trapping of basic drugs. If incorporated during melanin granule formation, they may become bound to melanin and this entrapment of

basic drugs maintains the diffusion gradient down which further drug can migrate by passive diffusion (Potsch et al., 1997). With its high melanin content, black hair concentrates basic drugs more effectively than white hair and brown hair is intermediate (Gaillard and Pepin, 1999). This is illustrated for the fluoroquinolone antimicrobial drug enrofloxacin and its metabolite ciprofloxacin in Fig. 3. Fig. 4 presents percentage binding versus concentration data for in vitro binding to melanin of six drugs. It will be seen that for the weak organic

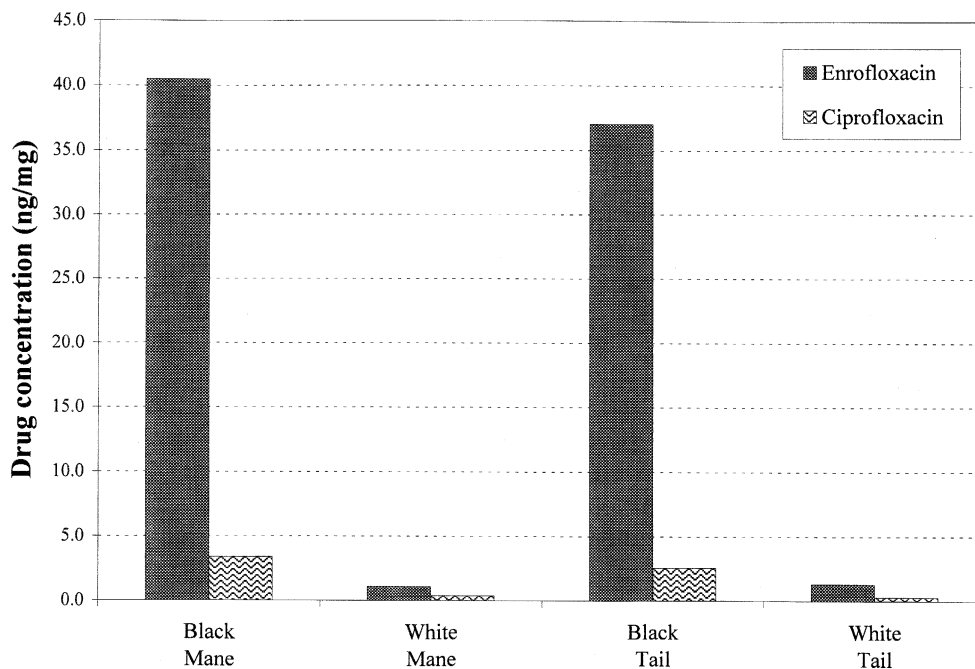


Fig. 3. In vivo uptake of the fluoroquinolone antibiotic enrofloxacin and its major metabolite ciprofloxacin in black and white equine mane and tail hair. Hair was collected from a single bi-coloured horse one month after oral administration of the drug at 5 mg/kg body weight for 10 days.

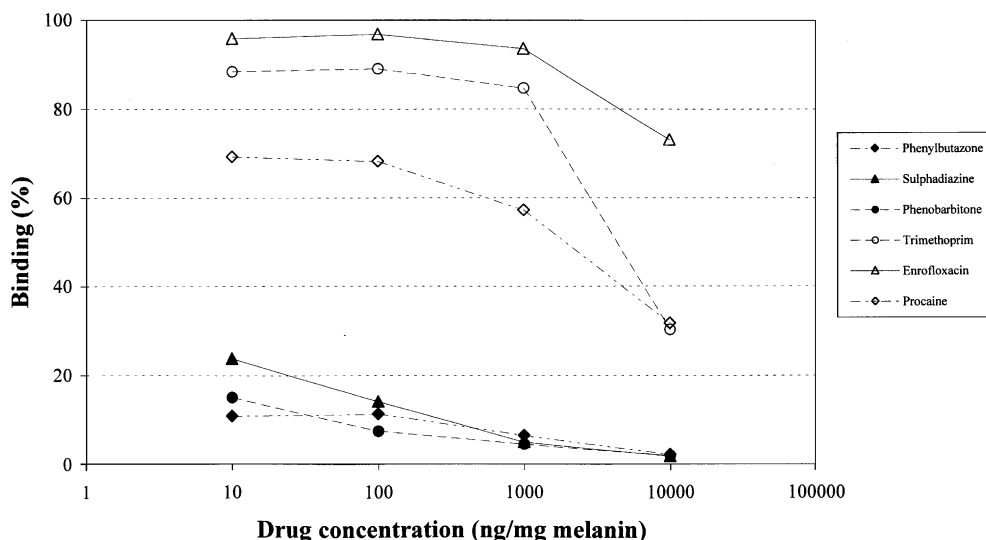


Fig. 4. In vitro drug-melanin binding of six drugs.

acids, phenylbutazone, sulphadiazine and phenobarbitone, binding is much lower than for the weak organic bases, trimethoprim and procaine. These data also indicate that binding becomes saturable at high drug concentrations, as reflected in a decrease in percentage binding. Melanin binding (affinity) has also been proposed as a possible explanation for the long-term retention of isoxsuprine in the horse as evidenced by the detection of this drug in post-race urine samples long after treatment was reported to have ceased (Torneke et al., 2000).

Nakahara (1999) proposed that the ratio, drug concentration in hair:plasma AUC, should be used as an index of drug incorporation tendency and as a basis for understanding incorporation mechanisms. For human hair the highest ratio was obtained for cocaine and the lowest was for 11-nor-tetrahydrocannabinol-9-carboxylic acid, the difference being 3600-fold. Current studies in our laboratory have established correlations between administered dose and hair concentration. This is illustrated for the organic bases procaine and trimethoprim and the organic acid sulfadiazine in Fig. 5. With in-

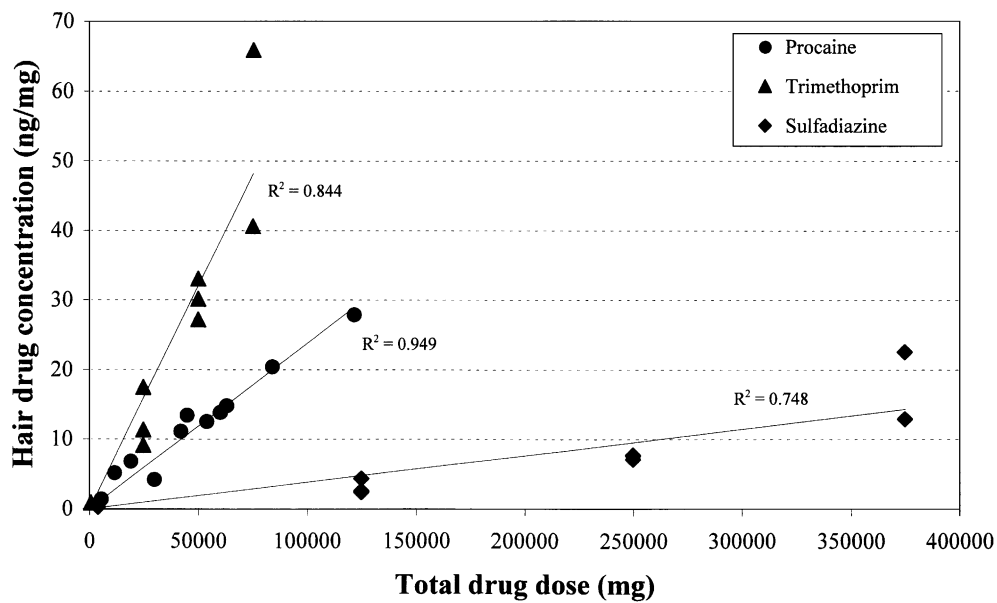


Fig. 5. Dose vs. hair drug concentration relationships for three drugs. Hair samples were collected up to three months post-clinical treatment with either intramuscular procaine benzylpenicillin or oral potentiated sulphonamides.

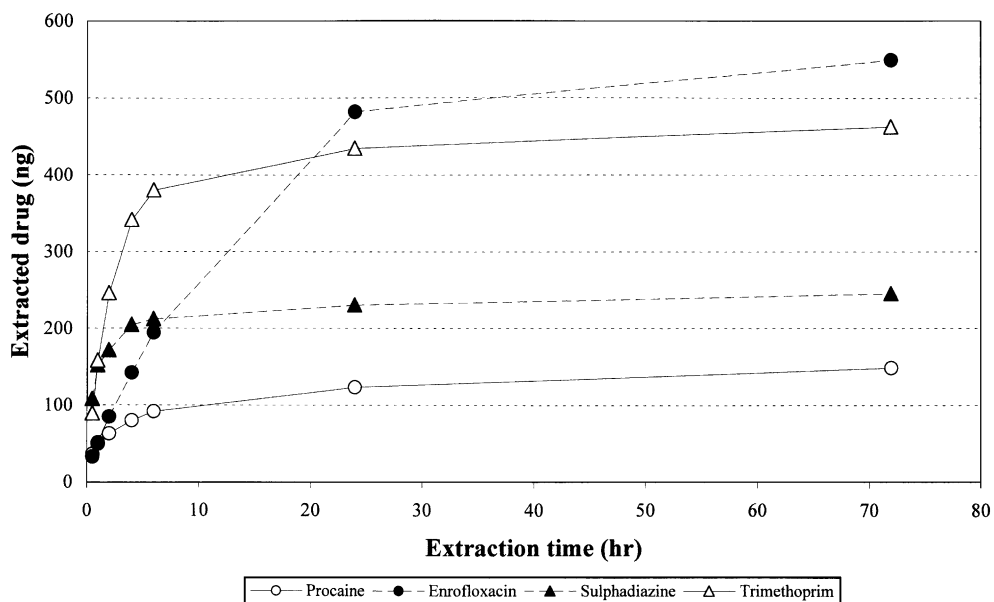


Fig. 6. Effect of incubation time on the extent of drug extraction. Samples of equine mane hair (approx. 20 mg) were incubated in 0.1 M hydrochloric acid at 65 °C.

creasing dose the highest hair concentrations were obtained for trimethoprim and the lowest concentrations for sulfadiazine.

6.3. Analytical methods

The principal analytical method of analysis used in our laboratory has been that of high pressure liquid chromatography, although for potent drugs, adminis-

tered in small amounts and present in hair in low concentrations, GC/MS methods may be required. Limited penetration into hair may also be associated with rapid clearance and short elimination half-life and with very high levels of binding to plasma protein, for example with NSAIDs.

For quantitative hair analysis, as well as requisite levels of sensitivity, chemical methods must be validated for accuracy and precision. Other important concerns

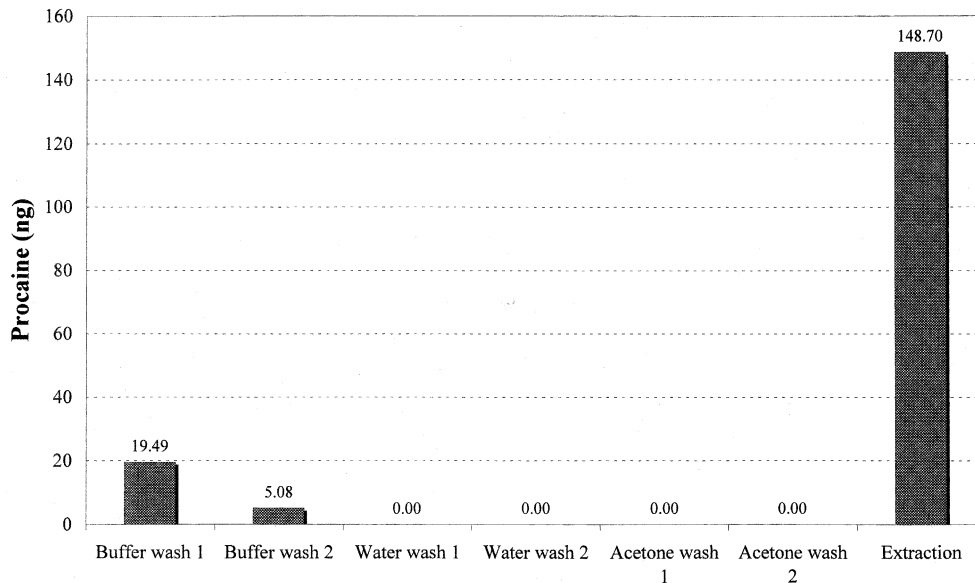


Fig. 7. Influence of successive washing procedures on extraction of procaine from equine hair.

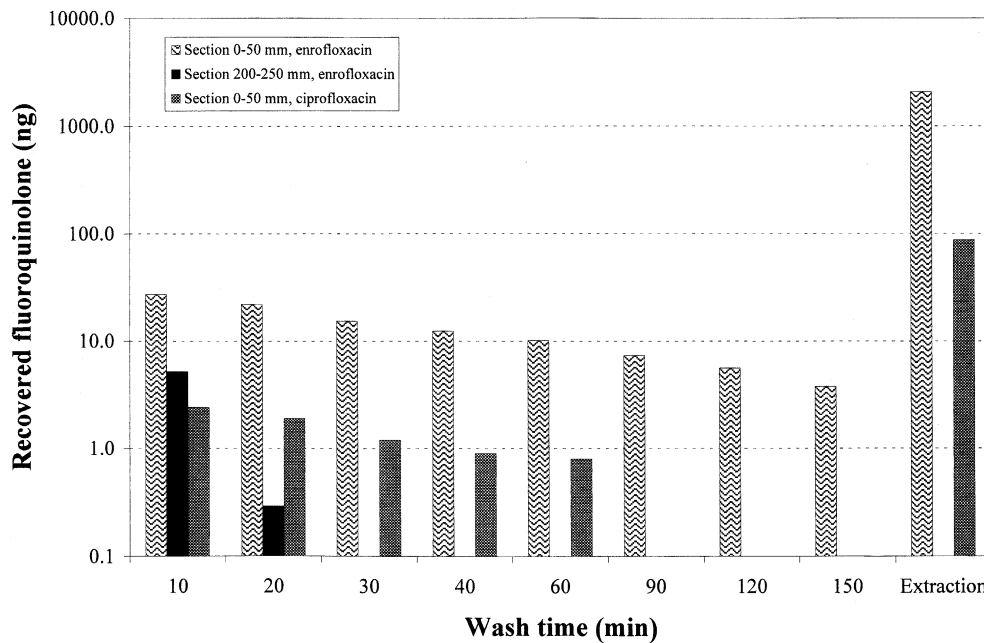


Fig. 8. Influence of wash time on recovery (expressed logarithmically) of enrofloxacin from two sections of equine hair (0–50 and 200–250 mm from the root) and of ciprofloxacin on one section of equine hair (0–50 mm from the root).

are extraction and clean up/washing procedures. The former must achieve a high and consistent percentage of drug present, whilst the latter must not only remove interfering substances but also allow distinction between drugs present as true excreta after systemic administration or ingestion and drug present as contaminant introduced by contact with urine, faeces or other environmental elements. The effect of incubation (extraction) time on amount of drug present in equine hair for four drugs is illustrated in Fig. 6. It will be seen that little further extraction is obtained when incubation exceeds 24 h. The influence of washing/extraction procedure on drug content of hair and amounts removed by washing is illustrated in Figs. 7 and 8. Fig. 7 illustrates losses of procaine arising from, successively, two buffer washes, two water washes and two acetone washes. The total loss was 16.5%. Fig. 8 illustrates the

effect of wash time on the recovery of enrofloxacin and ciprofloxacin from equine tail hair. It will be seen that losses were small in relation to amounts of the fluoroquinolones extracted.

6.4. Summary of findings and future prospects

To date, studies in our laboratory have shown that several anti-microbial drugs, including sulphonamides, trimethoprim, metronidazole (Fig. 9 illustrates the extraction of metronidazole from an extract of mane hair), enrofloxacin and procaine benzylpenicillin can be detected in mane and tail hair samples up to 2 years (and in one instance 3 years) after systemic administration. This implies virtually indefinite stability, although a steady decrease over time was reported by Dunnett et al. (2002a), after therapeutic administration of phenobar-

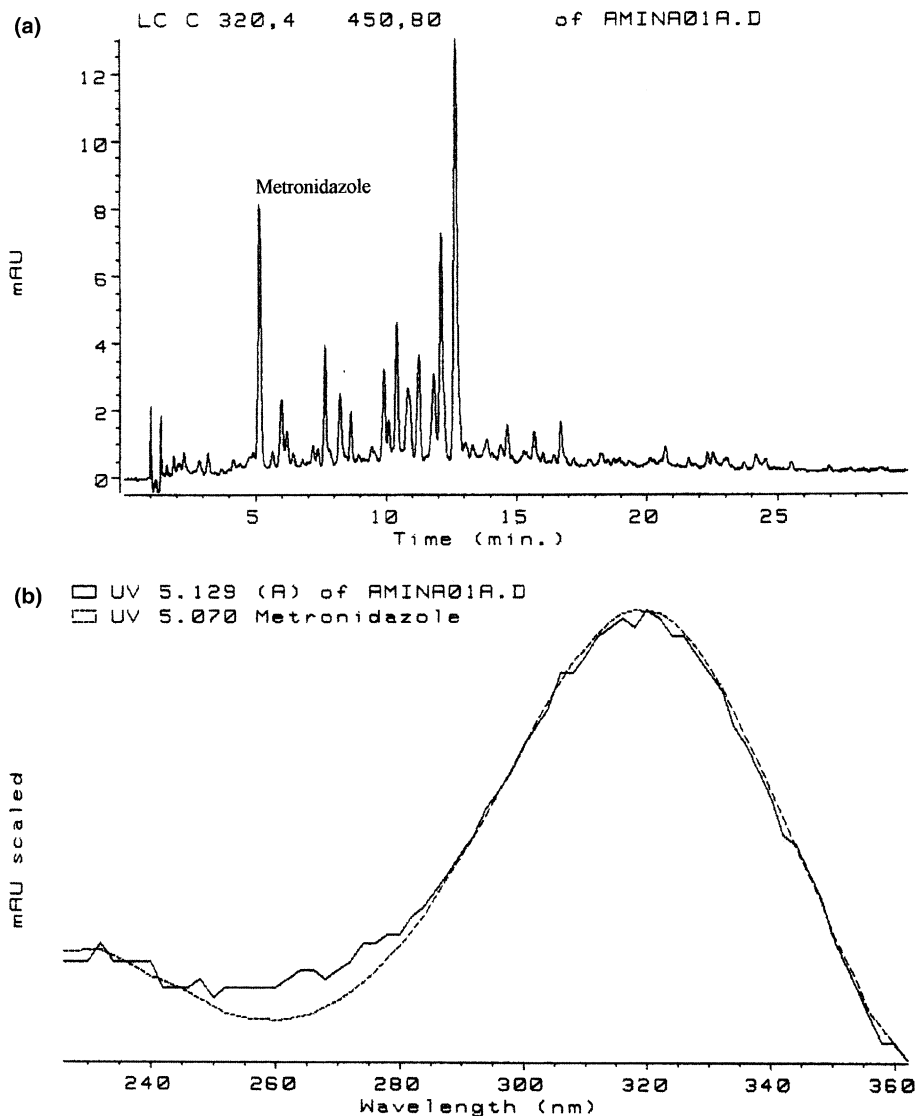


Fig. 9. Upper panel: Chromatogram showing the presence metronidazole in an extract of equine mane hair. Lower panel: Comparative UV spectra derived from the metronidazole peak in the analysed hair sample and that from a drug standard (Dunnett et al. (2003), reproduced with permission).

bitone to dogs for epilepsy management. Declining drug concentrations may be due to decomposition caused by heat or UV light or hair damage leading to drug leakage (Dunnett et al., 2002b; Dunnett and Lees, 2000). Further studies have also demonstrated the detection of several methylxanthine drugs, including caffeine and theobromine and their metabolites, in mane and tail hair (Dunnett et al., 2002b). Other investigators have detected performance-modifying drugs, including morphine (Beresford et al., 1998), diazepam and clenbuterol (Popot et al., 2000) in equine hair. However, cocaine was not detected in mane hair following systemic administration (Whittem et al., 2000). Studies in progress in our laboratory have extended drug analyses in hair and correlations between administered dose and drug pharmacokinetics to corticosteroids, NSAIDs and sedatives of various classes. It is clear that there are virtually no limitations to qualitative and quantitative analyses of drugs and their metabolites in equine hair.

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