THE DIAGNOSTIC POTENTIAL OF EQUINE HAIR: A COMPARATIVE REVIEW OF HAIR ANALYSIS FOR ASSESSING NUTRITIONAL STATUS, ENVIRONMENTAL POISONING, AND DRUG USE AND ABUSE

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Introduction

At first glance hair may appear to be perceived as a rather unprepossessing tissue; however, on closer scrutiny the truth is somewhat different. Early anatomical opinion considered the skin and hair as simply a passive barrier to fluid loss and mechanical injury. More recently, it has become apparent that the common integument of the skin and hair has to be regarded as a complex organ in which regulated molecular and cellular processes control essential physiological responses to environmental variables.

Hair has many functions, but most importantly it serves to help regulate body temperature and to provide a protective barrier against the horse's environment (Tregear, 1965). For example, there is a greater density of hair growth over those regions of the skin exposed to direct sunlight (Pilliner and Davies, 1996). Coat color is of some importance in thermal regulation, with light-colored coats being more effective in hot, sunny weather (Lyne and Short, 1965; Scott, 1988). Glossiness of coat hair is also important in reflecting solar radiation. Tropical breeds tend to have glossy coats that reflect solar radiation well (Hayman and Nay, 1961; Holmes, 1970). Equine skin carries several types of hair: temporary hair that comprises the majority of the coat; tactile hairs of the muzzle, ears, and eyes; and the permanent hair of the mane, tail, feathers, and eyelashes. These permanent hairs are anatomically located to provide protection in a number of ways. The mane helps to shed rainwater and to insulate the head and the major blood vessels to the brain (Pilliner and Davies, 1996), and the eyelashes protect against corneal impact injury. The various functions of hair are given in Table 1.

Table 1. Functions of hair (Stenn, 2001).

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

The Structure, Composition, and Growth of Hair

Structure of the hair shaft. The hair shaft is an end product of hair follicle growth and specialization and consists of three distinct structural components: a protective outer cuticle, an intermediately located cortex, and a central medulla (Harkey, 1993). A cross-sectional view of the hair shaft is shown in Figure 1. This illustrates the "tiled" structure of the overlapping cells of the outer cuticle that anchors the hair shaft to the follicle by interlocking with cells of the inner root sheath.

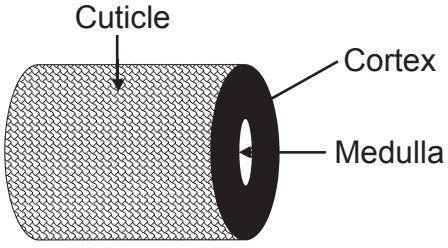

Figure 1. Cross section of the hair shaft.

The cortex constitutes the bulk of the hair shaft and comprises approximately 85% keratin, which is made up of matrix and fibrous proteins (Cone and Joseph, 1996). The protein fibers cross-link to form the structure of the hair and to provide its mechanical strength. The structural proteins of hair are interspaced with air gaps known as fusi. The cortex also contains melanin granules. Melanins (eumelanin and pheomelanin) are the pigments that give hair its color. The medulla is constructed of loosely packed, randomly orientated rectangular cells that shrivel when dehydrated, leaving a series of empty spaces (vacuoles) along the central axis of the hair shaft (Chatt and Katz, 1989). In general, the number of medulla cells, and thus the area of the medulla, increase with increasing hair fiber diameter. The fine hair of the equine coat contains predominantly cuticle and cortex cells, whereas the hair of the mane and tail contain a relatively large proportion of medulla cells (Talukdar et al., 1972; Harkey, 1993).

Chemical composition of the hair shaft. Hair is essentially a partially crystalline, cross-linked, and orientated polymeric protein structure. In addition to protein, the hair shaft comprises a number of different biochemical components including melanins, water, lipids, and inorganic minerals in variable amounts. Approximate proportions are given in Table 2.

Hair protein content derives primarily from the combination of three distinct structural keratins. These are designated as low-sulphur, high-sulphur, and high-tyrosine, high-glycine keratins. The sulphur content of hair is derived mainly from the proportion of sulphur-containing amino acids present, principally cysteine and to a lesser extent methionine. The lipid content of hair contains substances

Table 2. Approximate proportions of the various constituents of human hair

Constituent	*Proportion (%)*
Protein (keratins)	80-85
Water	<15
Lipids	1-9
Melanins	0.3-1.5
Inorganic minerals	0.25-0.95

such as free fatty acids and triglycerides. Melanins are polymeric substances produced from the oxidation of the amino acid tyrosine, through the action of the enzyme tyrosinase. In humans the melanin content of hair varies considerably between individuals and between races (Borges et al., 2001). The keratinized region of the hair shaft that extends beyond the epidermis of the skin (as the visible hair) is a dehydrated structure. The water content of the hair derives from atmospheric moisture and sweat and varies directly with the ambient relative humidity of the environment (Figure 2)(Robbins, 1979). Both trace elements and heavy metals such as lead, cadmium, and mercury can be found in hair. The concentrations of amino acid, lipids, and trace elements in hair are subject to variation due to factors such as genetics, diet, disease, environment, weathering, and cosmetic treatment.

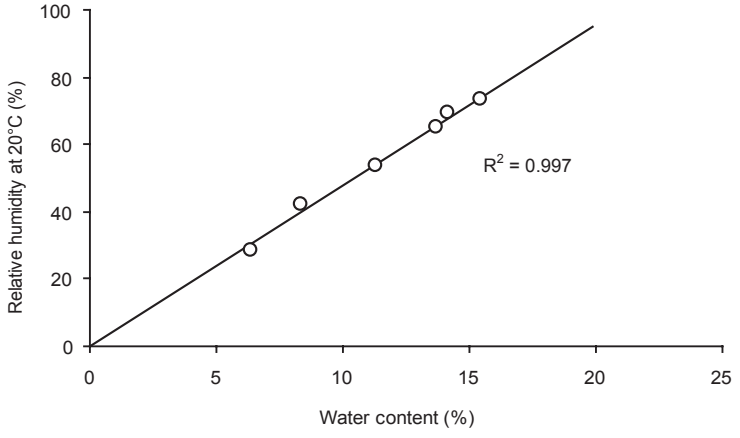

Figure 2. Relationship between hair water content and relative humidity.

Structure of the hair follicle. The hair follicle that produces the hair shaft is a miniature organ that contains muscular, vascular, and glandular components (Chatt and Katz, 1989). Follicles vary from site to site and generate hair shafts of varying shape, size, curl, and color depending on the anatomical location (Stenn and Paus,

2001). Unlike many species, including dogs and cats, that have compound hair follicles producing both primary and secondary hairs, the horse has simple follicles that generate only single hairs at any one time (Talukdar et al., 1972; Lloyd, 1993).

Simple follicles are associated with arrector pili muscles and both apocrine sweat and sebaceous glands. Contraction of the arrector muscle erects the hair shaft, influencing ventilation and heat loss from the skin, and is also associated with signaling in the fight-flight response to perceived danger. The sebaceous glands produce sebum, a lipid-based substance that coats the hair and skin to provide a protective barrier to repel water, to inhibit the growth of microorganisms (Lewis, 1995), and to prevent the penetration of toxic substances (Vale and Wagoner, 1997).

The follicle also comprises a dermal papilla, an inner and outer root sheath, and a bulge region (Figure 3). The dermal papilla directs the development of the follicular structure by supplying a permissive signal for continued growth of the hair. The hair bulb consists of proliferative epithelial cells that produce the inner and outer root sheaths and the hair matrix (Lloyd, 1993).

A further characteristic of the hair follicle as an organ is the presence of a range of enzyme systems that function to regulate the biochemical constitution of the tissue. The hair follicle contains a wide array of active enzyme systems including alcohol dehydrogenase, phosphorylase, NADPH reductase, glysosyltransferase, esterases, carboxylases, and succinic dehydrogenase (Jarrett, 1977; Potsch et al., 1997).

Hair growth cycle. The hair shaft develops via synthesis of matrix cells within the bulb. These cells move upwards and differentiate to form the various layers of the hair shaft and the surrounding root sheaths. When the hair shaft reaches the bulge area of the follicle, keratinization (hardening) occurs through protein cross-linking via formation of disulphide between adjacent cystine (amino acid) residues. The hair shaft then extrudes from the skin. The actual dynamics of hair growth is dependent on the rate of cell proliferation (Blume et al., 1991).

All mature hair follicles undergo a growth cycle comprising a period of active hair growth (anagen), a transitional period (catagen), a period of rest (telogen), and shedding (exogen) (Harkey, 1993; Lloyd, 1993; Stenn and Paus, 2001). The various stages of hair growth are shown in Figure 4, beginning with anagen in which the follicle is actively producing the hair shaft. During catagen active growth ceases and the follicle begins a shrinking process that ends in the telogen phase, where an inactive club hair is formed (Randall and Ebling, 1991).

At the end of the telogen phase, a new anogen phase begins. This is characterized by regeneration of the hair matrix from stem cells in the permanent part of the follicle, under the influence of the dermal papilla (Galbraith, 1998). A new hair shaft is then produced, the growth of which causes the previous club hair to be shed.

The duration of the hair growth cycle as a whole, and the duration of the individual phases within, varies between species, individuals, and anatomical sites. This cyclical activity is the mechanism by which animals change their pelage to meet the requirements of growth and seasonal climatic fluctuations (Randall and Ebling, 1991). It has also been proposed that the hair growth cycle protects against improper follicular formation and malignant degeneration (Stenn and Paus, 2001).

Hair Growth Rate in Horses: Non-Dietary Factors

The permanent hairs of the equine mane and tail undergo continual growth. Two studies involving small numbers of horses (four animals or less) over short periods of time suggest that the rate of growth of the mane is relatively constant (Whittem et al., 1998; Popot et al., 2000). In a much larger investigation involving 29 horses of different breeds, we found that mane and tail growth was essentially linear over a 12-month period (Figure 3). Although there was a degree of variability in the rates of hair growth in the mane and tail, when viewed on a month to month basis, no clear pattern was evident, and we were unable to correlate the fluctuations with climatic changes. The rate of growth varied between the mane and tail and within different regions of the mane (Figure 3). Rate of hair growth in the mane was lowest in the region near the withers and highest near the poll. The rates of growth of both the mane and tail were greater in native breeds of ponies than in Thoroughbreds, with crossbreds falling in between (Dunnett et al., 2002). We could find no clear effect of age or gender on hair growth rate in the mane or tail.

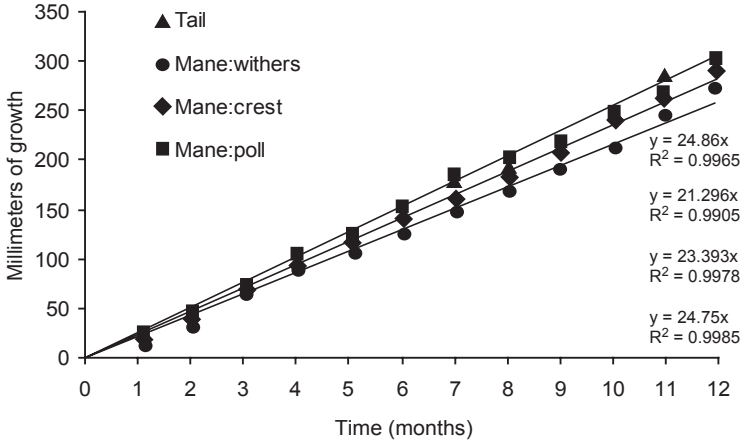

Figure 3. Cumulative mane and tail growth over time.

Effect of photoperiod. The seasonal growth and shedding of the pelage is something familiar to all owners of horses and domestic pets. Seasonal fluctuations in hair

growth in the dog have been studied by Butler and Wright (1981) and Gunaratnam and Wilkinson (1983). This phenomenon is common to wild and domesticated mammalian species. For example, hair growth is minimal or absent in winter in cattle (Dowling and Nay, 1960) and cats (Baker, 1974; Ryder, 1976). Wool growth in sheep reaches a maximum during summer or early autumn and is minimal in midwinter (Coop, 1953). Even in humans hair is subject to small but significant seasonal variation with hair growth peaking in late summer and early autumn (Randall and Ebling, 1991; Courtois et al., 1996). Changes in photoperiod during spring, late summer, and early autumn influence hair growth through the eyes, hypothalamus, hypophysis, pineal gland, thyroid gland, adrenal gland, and gonads. Growth also has an intrinsic or inherent cyclic rhythm, and the timing of the cycle can be altered by systemic factors such as hormonal changes. Although many exogenous and endogenous factors can affect the rate of hair cycling, none alter the sequence.

Equine pelage, like that in most mammals, is affected by changes in photoperiod. Time of onset and rate of coat shedding is increased in fillies exposed to artificially extended photoperiods (Wesson and Ginther, 1982) and mares (Oxender et al., 1977; Kooistra and Ginther, 1975). A study of seasonal changes in pony colts (Fuller et al., 2001) found that metabolic and pelage responses to photoperiod change were not immediate but lagged behind abrupt day length transitions by 5 to 8 weeks. Reports of seasonal changes in equine pelage indicate that the greatest rate of growth occurs during the autumn (Popot et al., 2000). Possible photoperiodic changes in the permanent hair of the mane and tail have yet to be investigated. Our own work has indicated that there may be a tendency for both mane and tail hair growth to increase during autumn; however, this apparent increase could not be statistically proven.

Melatonin. The effect of melatonin on mammalian pelage change is well-known. Light receptors in the eye ultimately relay changes in daylight length to the pineal gland, which synthesizes melatonin. As daylight decreases, melatonin synthesis increases and vice versa (Bergfelt, 2000).

Androgens. The influence of androgenic steroids such as testosterone on hair growth in horses has apparently not been investigated. Red deer stags, however, produce androgen-dependent long mane hairs during the breeding season (Thornton et al., 2001). Changes in circulating androgen levels in humans have also been postulated to affect hair growth, although this has not been proven (Randall and Ebling, 1991; Messenger, 1993).

Prolactin. Prolactin has been shown to affect hair follicle cycling in many mammals. Seasonal increases in circulating prolactin levels have been shown to be statistically related to shedding of the winter coat in male ponies (Argo et al., 2001).

Furthermore, administration of recombinant porcine prolactin to seasonally anestrous mares induced pelage shedding within 14 days (Thompson et al., 1997).

Thyroxine. Hair growth also appears to be influenced by systemic thyroxine levels. Increased thyroxine levels have been shown to stimulate hair growth in both humans (Parker, 1981) and dogs (Gunaratnam, 1986). Thyroxine deficiency has also been shown to be a common feature in diffuse alopecia (Ebling, 1981). There have been no extensive studies to investigate the effect of thyroxine levels on hair growth in horses; however, coarser coat hair growth occurs in thyroidectomized mares (Lowe et al., 1987).

Effect of climatic variables. Through the effect on melatonin and prolactin production, day length clearly affects pelage growth in mammals including horses, although no direct effect on equine mane and tail growth has been demonstrated. The effects of other climatic variables such as temperature, intensity of solar radiation, and relative humidity have not been extensively studied. Cold-housed young (7-month-old) Standardbred horses produced 1.4 to 2 times more coat hair than warm-housed horses of the same age and breed (Cymbaluk, 1990).

Dietary Factors Affecting Hair Growth Rate and Quality: Amino Acids, Lipids, Essential Elements and Trace Minerals, Vitamins, and Selenium

Hair follicles are metabolically active tissues that require nutrients to support both structural and functional activities (Galbraith, 1998). As such, nutrition has a profound effect on both its quality and quantity. Poor nutrition may produce, and therefore be reflected by, a dull, dry, brittle, or thin hair coat. Pigmentary disturbances may also occur. Nutritional factors that influence hair growth are very complex and can be interrelated. Those most commonly associated with poor hair quality and hair loss have been summarized by Lewis (1995). They comprise dietary deficiencies of protein, phosphorus, iodine, zinc, and vitamins A and E, as well as dietary excesses of selenium, iodine, and vitamin A. Other possible nutritional imbalances that can affect hair growth include B-vitamin and vitamin C deficiencies, copper and cobalt deficiencies, and molybdenum toxicosis (Scott, 1988).

Protein and amino acids

Hair is predominantly a protein-based tissue with a high percentage of sulphur-containing amino acid residues. The amino acid composition of hair protein has been extensively studied in many species, including humans (Robbins, 1979) and horses (Samata, 1985; Samata and Matsuda,1988). The most abundant amino

acids in equine hair are cystine, glutamic acid, serine, arginine, leucine, proline, and glycine. Samata and Matsuda (1988) observed differences in the amino acid content of equine hair between different breeds (Figure 4); however, no attempt was made to offer a possible explanation, and these apparent differences may have arisen due to methodological error or limited sample population. It was also reported that hair keratin amino acid content could be related to plasma amino acid levels, but no further details were offered (Samata and Matsuda 1988).

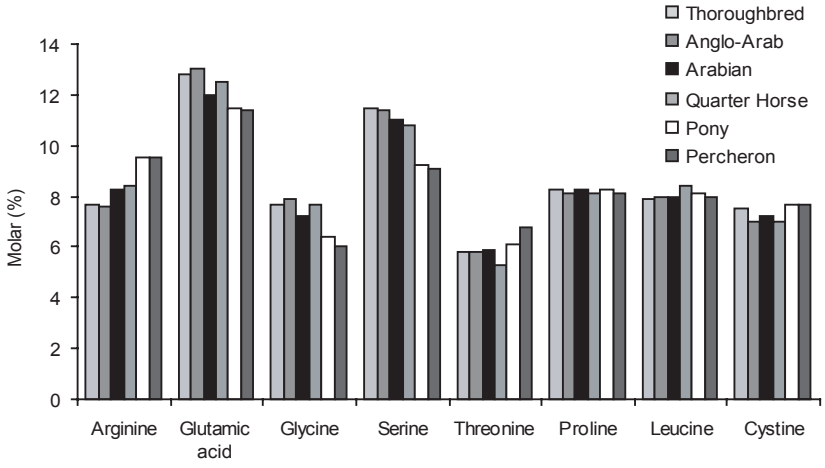

Figure 4. Differences in amino acid content in hair between equine breeds.

In mammals, normal hair growth and skin keratinization requires about 25% of an animal's daily protein requirement (Scott, 1988; Muller, 1989). Consequently, protein deficiency arising through starvation, low protein diet, or chronic catabolic disease results in hair production of abnormal texture and decreased length and diameter. In more extreme instances, diffuse thinning of the coat or alopecia can occur (Scott, 1988). Protein deficiency causes hair growth and shedding to be slowed (Lewis, 1995). The most important requirement for hair keratin synthesis is the sulphhydryl-containing amino acid cysteine, as it is ultimately oxidized to form the stable disulphide bonds that give keratin its structure, strength, and stability. Keratin is approximately 4% sulphur. Highest hair cysteine concentrations are found in the cuticle and cortex, and the lowest in the root sheaths (Rook and Dawber, 1982). Horses, like nonruminants, are unable to absorb inorganic sulphur and must meet their sulphur requirements through organic forms, such as the preformed sulphur-containing amino acids in plants: methionine, cysteine, and cystine (Lewis, 1995). Methionine can be converted to cysteine in the liver, and cystine is reduced to cysteine in the liver and blood. Despite the popularity of supplemental organic forms of sulphur, such as methylsulphonylmethane (MSM),

there appear to be no reported specific sulphur deficiencies in the horse that could impair keratin synthesis. Whether sulphur supplementation (via sulphur-containing amino acids or MSM) and/or supplementation with other amino acids in horses with adequate dietary supplies can improve hair quality is uncertain. In other species, particularly sheep, increases in dietary sulphur content can, within certain limits, increase sulphur amino acid content in hair and can lead to increased hair growth rate and quality.

Lipids

The lipid content of hair is comprised of free fatty acids, monoglycerides, diglycerides, triglycerides, wax esters, hydrocarbons, and alcohols predominantly derived from surface deposition of sebum and apocrine secretions (Chatt and Katz, 1989). However, a proportion of hair lipid has been shown to form an integral part of the hair structure in humans. These integral hair lipids include cholesterol sulphate and ceramides (similar to those found in the keratinized portion of the epidermis), in addition to cholesterol, fatty alcohols, and free fatty acids. The predominant fatty acid, comprising 40% of the total fatty acids in the integral lipid faction, was identified as 18-methyl-eicosanoic acid (Wertz and Downing, 1988). Sebum and apocrine secretions normally coat the hair. These secretions of the sebaceous and apocrine glands are necessary to form a barrier to repel water and to protect against infection from microorganisms (Baxter and Trotter, 1969). Chronic illness may induce decreased production of sebum and apocrine secretions, resulting in dry lusterless hair. Such poor hair quality, in addition to hair thinning or alopecia, can also arise from essential fatty acid deficiency. Linoleic acid is an essential fatty acid needed for sebum production. Although essential fatty acid deficiencies are seen in dogs (Muller, 1989) and pigs (Scott, 1988), this condition does not appear to occur in horses (Lewis, 1995).

There has been a widely held belief that fat supplementation, particularly with oils rich in linoleic acid, will increase the quantity of sebum produced and thus lead to hair with a glossier appearance. Conclusive data to support this assumption have been lacking; however, Harris et al. (1998) observed significant improvements in coat, mane, and tail appearance (gloss and softness) following fat supplementation. There was also reportedly a significant increase in sebum and apocrine-secretion production. Recently, it has been demonstrated by administration of radio-labelled free fatty acids that dietary linoleic and linolenic acids are incorporated into guinea pig hair, presumably via sebum (Fu et al., 2001).

Carbohydrates

The main energy source for the hair follicle is glucose. During anagen the outer root sheath of the follicle is rich in glycogen, the storage form of glucose. This

glycogen reserve disappears during catagen and is absent during the telogen phase (Jarrett, 1977; Rook and Dawber, 1982).

Essential elements and trace minerals

Few essential elements and trace minerals appear to have significant and clearly defined roles in relation to hair growth and quality. Those that do include iodine, zinc, and copper.

Iodine. This element is essential for the production of thyroxine. The effects of thyroxine on hair growth and quality have been discussed earlier. Iodine deficiency produces diffuse alopecia with premature telogenesis and hypoplastic hair follicle in all domestic species (Scott, 1988; Muller, 1989).

Zinc. Zinc is an essential element to many metalloenzymes and metabolic processes, including keratogenesis. It is also a cofactor for RNA and DNA polymerases and is involved in the synthesis of free fatty acids and vitamin A metabolism. Deficiency of zinc through low-zinc diets, high-calcium diets, phytates, or other chelators have been reported to cause alopecia in several species including dogs, goats, cattle, sheep, and pigs (Scott, 1988; Muller, 1989). There appear to be no reports of clinical zinc deficiency in horses, but experimentally induced zinc deficiency leads to hair loss (Harrington et al., 1973).

Copper. This element is essential in various enzyme systems including those involved in melanin synthesis, keratin synthesis, and disulphide bond linkage (Jarrett, 1977; Underwood, 1977). Copper deficiency results in fiber depigmentation and loss of hair tensile strength and elasticity leading to breakage. However, copper deficiency has not been observed in horses.

Molybdenum. Although not involved in keratogenesis and hair growth, excessive dietary levels of molybdenum can cause reduced hair growth and loss in hair quality. This is not a direct effect of molybdenum on hair but arises from interference with hepatic storage of copper and copper deficiency. However, there appear to be no reported cases of molybdenum toxicosis in horses.

Vitamins

Vitamins are essential to the promotion and regulation of a multitude of vital physiological and metabolic processes common to many organ systems in the horse.

Consequently, any disruption to the dietary supply or in vivo synthesis of vitamins has the potential to affect hair growth to a greater or lesser degree. In

practice vitamin deficiencies are rare causes of disturbed hair growth in domestic mammals, including horses (Scott, 1988; Muller, 1989).

Vitamin A and ß-carotene. Vitamin A is formed in the small intestine primarily from the enzyme-catalyzed cleavage of dietary ß-carotene. Vitamin A is required for healthy epithelial tissue formation through participation in glycoprotein synthesis that controls cell differentiation and gene expression. Correct cell differentiation is essential for the production of healthy inner and outer root sheath and hair matrix cells. Vitamin A deficiency can occur in foals and foaling mares. This results in rough, dry, dull, brittle, and long coat hair in both foals and mares (Donoghue et al., 1981; Evans et al., 1977). Similar coat hair defects are also observed in vitamin A toxicosis (Donoghue et al., 1981). More recently, in vitro experiments have shown that a vitamin A analogue, 13-cis-retinoic acid, alters equine hair sheath-shaft interactions. It was further hypothesized that in vivo hair sheath growth may be mediated by the follicle at the level of the sebaceous gland or by the sebaceous gland itself through the action of a biochemical factor analogous with vitamin A (Williams et al., 1996). Interestingly, some ß-carotene not cleaved in the gastrointestinal tract is absorbed and transported, bound to HDL lipoproteins, to various tissues, including the skin and corpus luteum. There are reported to be a number of poorly understood intrinsic mechanisms that regulate hair growth (Scott, 1988), and it is therefore possible that ß-carotene in the skin may directly affect hair growth by some unknown mechanism. Alternatively, ß-carotene could influence hair growth indirectly through its involvement in progesterone secretion from the corpus luteum.

B-complex vitamins. Since many of the B vitamins are involved in regulation of energy metabolism and protein synthesis, their deficiencies can lead to decreased feed intake, inadequate protein supply, increased protein catabolism, reduced keratogenesis, and therefore impaired hair growth and shedding. Extreme biotin deficiencies induced experimentally in a number of species, but not horses, resulted in hyperkeratosis of the follicular epithelia, alopecia, and depigmentation of the hair (Grieve, 1963; Misir et al., 1986). Biotin-containing supplements are widely marketed for promoting growth of equine hoof horn, a keratin-containing tissue. There is some evidence to suggest that biotin administration improves hair growth in swine (Bryant et al., 1985) but there appears to be no similar scientific evidence pertaining to its administration in horses. Furthermore, there is currently no clinical evidence for biotin deficiency in horses.

Vitamin C. Ascorbic acid (vitamin C) is important for hair growth and stability through facilitating the formation of disulphide linkages in keratin, and a vitamin C-responsive alopecia in recognized in calves (Goldsmith, 1983; Scott, 1988). No similar condition has been reported in horses. Adequate supply of ascorbic acid is

also necessary for synthesis of both steroid hormones and hydroxyproline. Steroids, such as the androgens, are suspected of having a regulatory role in hair follicle cycling. Hydroxyproline is a major constituent of collagen and therefore connective tissue. Normal collagen is essential for the structural integrity of inner and outer hair root sheaths.

Vitamin E. This vitamin, in conjunction with selenium, provides the natural antioxidant system that helps to maintain cell membrane stability. Vitamin E functions as a free radical scavenger that protects cells from damage arising from reactive oxygen species (radicals), a major source of which is lipid metabolism. A high fat diet can lead to relative vitamin E deficiency. Subsequent free radical production and resultant membrane damage can lead to loss of cellular integrity and hair loss.

Selenium

Selenium performs a number of roles pertaining to cellular function and is a necessary constituent of the diet. However, chronic toxicity occurs when dietary levels exceed 5 mg/kg, and acute toxicity evolves when levels reach 25-50 mg/kg. Toxic selenosis occurs occasionally in horses (Crinion and O'Connor, 1978; McLaughlin and Cullen, 1986; Dewes and Lowe, 1987; Witte et al., 1993). In this condition it is assumed that selenium substitutes for sulphur in sulphur-containing amino acids forming selenomethionine, selenocysteine, and selenocystine. Incorporation of absorbed seleno-amino acids during keratogenesis results in reduced capacity to form disulphide cross-links causing reduced structural integrity. Selenosis in horses generally occurs in areas of high soil selenium content and develops through chronic ingestion of selenium-concentrating plants and water of high selenium content. The condition results in progressive loss of hair from the mane, tail, and fetlocks and in extreme cases a generalized alopecia.

Hair Analysis: An Historical Perspective

Hair analysis was first applied in the middle of the 19th century. Casper (1857-1858) recorded its use to detect the presence of arsenic in hair from a suspected murder victim 11 years postmortem. The technique then appears to have been dormant for almost 100 years until in 1945 Flesch (1945) proposed that hair might be considered a metabolic end product and excretory organ, the trace element composition of which reflected the medium from which it was formed.

Early values for metal concentrations in hair were reported by Goldblum et al. (1953). A year later, Goldblum et al. (1954) described the detection of the first organic drug, phenobarbitone, in hair. Forshufvud et al. (1961) and Smith et al. (1962) used hair analysis to investigate suspicions that the Emperor Napoleon had

been poisoned with arsenic. The results of the analysis indicated repeated exposure to arsenic. No firm conclusion as to the source of the arsenic could be drawn as it appears that pigments based on arsenic were used in wallpaper manufacture in the early 19th century.

The application of hair analysis to monitor exposure to heavy metals and nutritional trace elements continued through the 1960s and 1970s. The theory that hair could be used as a means to identify and track prior exposure to heavy metal toxins was explored and exploited with varying degrees of success in diverse situations. Hair analysis was used to trace the history and extent of exposure to mercury in cases of suspected poisoning in Iraq arising from consumption of bread produced from grain contaminated with mercurial fungicide. Other uses of the technique were to monitor occupational and lifestyle exposures to heavy metal toxins, such as mercury in dental technicians and lead from traffic exhaust emissions in school children.

The application of hair analysis for the detection of abuse of controlled drugs and to establish an individual's history of use was initiated by Baumgartner et al. (1979), who applied the technique to the detection of opiates in samples from drug addicts. Over the next decade, the application of the technique was expanded to encompass a range of drugs of abuse, including phencyclidine (Baumgartner et al., 1981), barbiturates (Smith and Pomposini, 1981), and cocaine (Valente et al., 1981). The subsequent 20 years has seen an exponential increase in the development, validation, and application of hair analysis to the detection of a wide range of abused and therapeutic drugs in human hair (Tagliaro et al., 1997; Nakahara, 1999; Gaillard and Pepin, 1999).

Hair Analysis to Assess Nutritional Status

Hair analysis for assessment of nutritional status (for essential elements and trace minerals) has been evaluated and employed for several decades. However, it is only within the last 10 to 20 years that increasing use of spectroscopic methods, making multi-element analysis possible, and improvements in analytical methodologies have provided a more reliable, rapid, and comparatively inexpensive diagnostic technique (Chyla and Zyrnicki, 2000). Hair is a potentially useful tissue for trace element analysis insofar as it is easily collected and transported, has high concentrations of trace elements, and may be representative of nutritional status over an extended time period. This last point may provide a benefit over plasma and urine samples, as transient fluctuations arising from recent dietary intake may be avoided.

Hair analysis has been used in the past in attempts to measure whole-body status of trace minerals such as calcium and phosphorus (Sippel et al., 1964; Wysocki and Klett, 1971); copper, molybdenum and iron (Cape and Hintz, 1982); and zinc, copper, and selenium (Wichert et al., 2002). However, the potential

benefits of hair analysis can only be realized if measured hair concentrations are indicative of whole-body status and can accurately and consistently reflect nutritional imbalances. To date, there remain questions over the validity of hair analysis for the assessment of whole-body trace element status in horses (Hintz. 2000).

Monitoring Heavy Metal Exposure and Other Environmental Toxins

Hair analysis has been utilized extensively to investigate human exposure to a number of toxic heavy metals and other elements including lead, cadmium, mercury, and arsenic, and to track the history of such exposure. This topic has been reviewed extensively by Chatt and Katz (1989). The technique has been much less widely applied in animal toxicological studies, although it has been used to identify environmental exposure to heavy metals (Burger et al., 1994) and selenium (Clark et al., 1989; Edwards et al., 1989) in wildlife, and selenosis in domestic animals (Mihajlovic, 1992). Levine et al. (1976) reported that lead levels in blood and coat hair from a number of dead small and large animals that had been grazing pasture near a lead smelter were significantly elevated.

Environmental exposure of horses to a number of toxic elements has been investigated by hair analysis. Cadmium exposure of horses in central Europe in relation to their age, gender, breed, and location was examined by hair analysis. Cadmium was detectable in hair samples and was accumulated to a greater extent in geldings than mares (Anke et al., 1989). Hair analysis was used to monitor exposure of horses, sheep, and alpacas to a number of toxic heavy metals and other elements including cadmium, chromium, nickel, and bromine in vehicle emissions (Ward and Savage, 1994). Elevated lead and cadmium levels were found in horse hair and blood samples. Lead contents in blood and hair were significantly correlated ($r = +0.69$). Exposure of horses to toxic levels of selenium via forage ingestion has been investigated by hair analysis. Hair selenium concentrations in coat, mane, and tail hair samples ranged from 0.3–7.1 mg/kg. Hair selenium concentrations were strongly correlated with serum concentrations ($r = 0.76$–0.94) (Witte et al., 1993). Hair selenium concentrations are partially cumulative and reflect historical exposure to this element.

Dauberschmidt and Wennig (1998) used hair analysis to investigate pesticide exposure in humans. DDE and other polychlorinated biphenyls (PCB) were detected at concentrations of 0.5-4.9 pg/mg. As yet, assessment of the exposure of horses to environmental pesticide residues by hair analysis has not been attempted.

Another unevaluated potential application of hair analysis is in instances of plant-induced toxicosis in horses. Although plant poisoning in horses continues to be a problem in many countries throughout the world, its economic impact is unclear, as comparatively few poisonings are confirmed, and the actual extent of

the problem is not clearly known. In the UK, however, approximately 500 horses each year die from hepatic disease resulting from the ingestion of common (or tansy) ragwort. The hepatotoxins in this plant are a group of substances known as pyrrolizidine alkaloids. In addition to causing primary hepatic failure, these substances are also carcinogenic and teratogenic (Lewis, 1995). These substances, in common with other alkaloids present in many other plants, should be chemically amenable to deposition in equine hair and therefore subsequent detection. We are currently developing analytical techniques to detect and identify alkaloid plant toxins in equine hair.

Hair Analysis and Drug Use: Residue Monitoring in Stock Production, Pre-Purchase Examinations, and Sports Anti-Doping Control

The use of hair analysis for the detection of drug administration in horses has only begun to be evaluated within the last 5 years. Horses can be subject to drug abuse in several instances. The growth-promoting potential of anabolic steroids such as testosterone, nandrolone, and stanozolol, and repartitioning agents such as clenbuterol (Ventipulmin), albuterol, and brombuterol, can be abused during bloodstock breeding to enhance muscular development of young horses. A multitude of performance-altering drugs including central nervous system stimulants and sedatives have been and continue to be misused during training and competition in equine sports such as racing and show jumping. Nonsteroidal anti-inflammatory analgesic drugs such as phenylbutazone (bute) and anti-inflammatory corticosteroids including dexamethasone are known to be used to mask lameness in horses during pre-purchase veterinary examinations. Furthermore, in the European Union many therapeutic drugs intended for use in equine veterinary medicine are no longer permitted to be used for treatment in horses intended for human consumption.

In 1997 we began a long-term investigation to study the incorporation of therapeutic and illicit drugs in equine mane and tail hair and to evaluate the potential for hair analysis for the retrospective detection of drug use and misuse in horses. We have shown that a number of antibiotics including sulphonamides, trimethoprim, metronidazole, and procaine benzylpenicillin can be detected in mane and tail hair samples up to two years after systemic administration (Dunnett and Lees, 2000; Dunnett et al., 2002). We have also detected a number a range of methylxanthine drugs and metabolites including caffeine and theobromine in mane and tail hair (Dunnett et al., 2002). Deposition of a number of other drugs in equine hair has recently been reported, including morphine (Beresford et al., 1998), diazepam, and clenbuterol (Popot et al., 2000). Cocaine, however, was not detected in mane hair following systemic administration (Whittem et al., 2000).

Thoroughbred racehorses can fail postrace drug tests for prohibited substances through ingestion of a number of potential feed contaminants. Some examples of prohibited substances that can occur in feedstuffs are shown in Table 3.

Table 3. Examples of prohibited substances that occur in feedstuffs.

Arsenic	Hyoscine
Atropine	Lupanine
Borneol	Menthol
Bufotenine	Morphine
Caffeine	Oryzanol
Camphor	Sparteine
Dimethylsulphoxide	Theobromine
Hordenine	

It is possible that the application of hair analysis provides additional analytical evidence to that normally achieved from blood or urine analysis. Analysis of hair, unlike that of urine and blood, may demonstrate that the presence of the drug in the horse's system arose from the chronic ingestion of contaminated feed rather than from an acute drug administration. The potential routes for the incorporation of drugs and other substances, including trace minerals, into hair is shown in Figure 5.

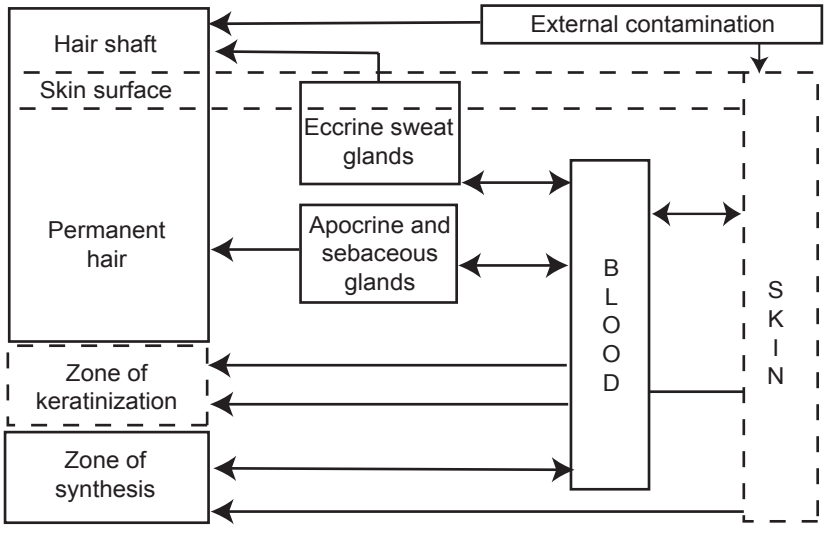

Figure 5. Potential routes of incorporation of drugs and other substances into hair (Henderson,1993).

References

Anke, M., T. Kosla, and B. Groppel. 1989. The cadmium status of horses from central Europe depending on breed, sex, age and living area. Arch. Tierernahr. 39:657-683.

Argo, C.M., M.G.R. Collingsworth, and J.E. Cox. 2001. Seasonal changes in reproductive and pelage status during the initial quiescent and first active breeding seasons of the peripubertal pony colt. Anim. Sci. 72:55-64.

Baker, K.P. 1974. Hair growth and replacement in the cat. Br. Vet. J. 130:327-335.

Baumgartner, A.M., P.F. Jones, W.A. Baumgartner, and C.T. Black. 1979. Radioimmunoassay of hair for determining opiate-abuse histories. J. Nucl. Med. 20:748-752.

Baumgartner, A.M., P.F. Jones, and C.T. Black. 1981. Detection of phencyclidine in hair. J.Forensic Sci. 26:76-581.

Baxter, M., and D. Trotter. 1969. The effect of fatty materials extracted from keratins on the growth of fungi, with particular reference to the free fatty acid content. Sabouraudia 7:199-206.

Beresford, G.D., T.A. Gourdie.and E. Whittem. 1998. Analysis of morphine in equine hair samples by GC/MS. Proc. 13th Internat. Conf. of Racing Analysts and Veterinarians. Vancouver, BC, Canada.

Bergfelt, D.R. 2000. Anatomy and physiology of the mare. In: Equine Breeding Management and Artificial Insemination. p. 141-164. W B Saunders, London.

Blume, R.A., J. Ferracin, M. Verschoore, J.M. Czernielewski, and H. Schaefer. 1991. Physiology of the vellus hair follicle: Hair growth and sebum excretion. Br. J. Dermatol. 124:21-28.

Borges, C.R., J.C. Roberts, D.G. Wilkins, and D.E. Rollins. 2001. Relationship of melanin degradation to actual melanin content: Application to human hair. Anal Biochem. 290:116-125.

Bryant, K.L., E.T. Kornegay, and J.W. Knight. 1985. Supplemental biotin for swine III. J. Anim. Sci. 60:154-162.

Burger, J., M. Marquez, and M. Goechfeld. 1994. Heavy metals in the hair of opossum from Palo Verde, Costa Rica. Arch. Environ. Contam. Toxicol. 27**:**154-161.

Butler, W.F., and A.I. Wright. 1981. Hair growth in the greyhound. J. Small Anim. Pract. 22**:**655-661.

Cape, L., and H.F. Hintz. 1982. Influence of month, colour, age, corticosteroids and dietary molybdenum on mineral concentration of equine hair. Am. J. Vet. Res. 43:1132-1136.

Casper, J.L. 1857-1858. Praktisches handbuch der gerichtlichen medizin, (2 vol.). A. Hirschwald, Berlin.Chatt, A., and S.A. Katz. 1989. Hair Analysis:

Applications in the Biomedical and Environmental Sciences. VCH Publications, New York.

Chyla, M.A., and W. Zyrnicki. 2000. Determination of metal concentrations in animal hair by the ICP method. Comparison of various washing procedures. Biol. Trace Element Res. 75:187-194.

Clark, D.R., P.A. Ogasawana, G.J. Smith, and H.M. Ohlendorf. 1989. Selenium accumulation by raccoons exposed to irrigation drain water at Kesterson National Wildlife Refuge, California, 1986. Arch. Environ. Contam. Toxicol. 18:789-794.

Cone, E.J., and R.E. Joseph. 1996. The potential of bias in hair testing for drugs of abuse. In: Drug Testing in Hair. p. 69-93. CRC Press, London.

Coop, I.E. 1953. Wool growth as affected by nutrition and climate factors. J. Agric. Sci. 43:456-463.

Courtois, M., G. Loussouarn, S. Horseau, and J.F Grollier. 1996. Periodicity in the growth and shedding of hair. Br. J. Dermatol. 134:47-54.

Crinion, R.A.P., and J.P. O'Connor. 1978. Selenium intoxication in horses. Irish Vet. J. 30:81-86.

Cymbaluk, N.F. 1990. Cold housing effects on growth and nutrient demand of young horses. J. Anim. Sci. 68:3152-3162.

Dauberschmidt, C., and R. Wennig. 1998. Organochlorine pollutants in human hair. J. Anal. Toxicol. 22:610-611.

Dewes, H.F., and M.D. Lowe. 1987. Suspected selenium poisoning in a horse. N. Z. Vet. J. 35:53-54.

Donoghue, S., D.S. Kronfeld, and S.J. Berkowitz. 1981. Vitamin A nutrition of the equine. J. Nutr. 111:365-374.

Dowling, D.F., and T. Nay. 1960. Cyclic changes in the follicles and hair coat in cattle. Aust. J. Ag. Res. 11:1064-1071.

Dunnett, M., E. Houghton, and P. Lees. 2002. Deposition of etamiphylline and other methylxanthines in equine mane hair following oral administration. Proc. 14th Internat. Conf. of Racing Analysts and Veterinarians. R & W Publications, Orlando, Florida.

Dunnett, M., and P. Lees. 2000. Hair analysis as a novel investigative tool for the detection of historical drug use/misuse in the horse. Proc. 8th Internat. Congr. of the European Assoc. of Veterinary Pharmacologists and Toxicologists, Jerusalem, Israel.

Ebling, F.J. 1981. Hormonal control of hair growth. In: C.E.O. Orfanos, G.S. Montagna, and G.S. Stuttgen (Eds.) Hair Research. pp. 195-204. Springer-Verlag, Berlin.

Edwards, W.C., D.L. Whitenack, J.W. Alexander, and M.A. Solangi. 1989. Selenium toxicosis in three California sealions (*Zalophus californianus*). Vet. Hum. Toxicol. 31:568-570.

Evans, J.W., A. Borton, H.F. Hintz,.and D.L. Van Vleck. 1977. The Horse. W H Freeman and Co., New York.

Flesch, P. 1945. Physiology and Biochemistry of the Skin. S. Rothman (Ed.). pp. 601-661. University of Chicago Press, Chicago.

Forshufvud, S., H. Smith, and A. Wassen. 1961. Nature 192:103-105.

Fu, Z., N.M. Attar-Bashi, and A.J. Sinclair. 2001. 1-14C-linoleic acid distribution in various tissue lipids of guinea pigs following an oral dose. Lipids 36:255-260.

Fuller, Z., J.E. Cox, and C.M. Argo. 2001. Photoperiod entrainments of seasonal changes in the appetite, feeding behaviour, growth-rate and pelage of pony colts. Anim. Sci. 72:65-74.

Gaillard, Y., and G. Pepin. 1999. Testing hair for pharmaceuticals. J. Chromatogr. B. Biomed. Sci. Appl. 733**:**231-246.

Galbraith, H. 1998. Nutritional and hormonal regulation of hair follicle growth and development. Proc. Nutr. Soc. 57**:**195-205.

Goldblum, R.W., S. Berby, and A.B. Lerner. 1953. The metal content of skin, nails and hair. J. Invest. Dermatol. 20:13.

Goldblum, R.W., L.R. Goldbaum, and W.N. Piper. 1954. Barbiturate concentrations in the skin and hair of guinea pigs. J. Invest. Dermatol. 22**:**121-128.

Goldsmith, L.A. 1983. Biochemistry and Physiology of the Skin. Oxford University Press, Oxford.

Grieve, J.H. 1963. Effects of thyroid and biotin deficiences on canine demodicosis. Diss. Abstr. 24:1757.

Gunaratnam, P. 1986. Effects of thyroxine on hair growth in the dog. J. Small Anim. Pract. 27**:**17-29.

Gunaratnam, P., and G.T. Wilkinson. 1983. A study of normal hair growth in the dog. J. Small Anim. Pract. 24:445-453.

Harkey, M.R. 1993. Anatomy and physiology of hair. Forensic Sci. Int. 63**:**9-18.

Harrington, D.D., J. Walsh, and V. White. 1973. Clinical and pathological findings in horses fed zinc deficient diets. Proc. Equine Nutr. and Physiol. Soc. Symp., p. 51.

Harris, P.A., J.D. Pagan, K.G. Crandell, and N. Davidson. 1998. Effect of feeding thoroughbred horses a high unsaturated or saturated vegetable oil supplemented diet for 6 months following a 10 month fat acclimation. Proc. 5th Internat. Conf. on Equine Exercise Physiol., Utsunomiya, Japan.

Hayman, R.H., and T. Nay. 1961. Observation on hair loss and shedding in cattle. Aust. J. Agric. Res. 12:513-527.

Hintz, H.F. 2000. Hair analysis as an indicator of nutritional status. J. Equine Vet. Sci. 21**:**199.

Holmes, C.W. 1970. Effects of air temperature on body temperatures and sensible heat loss of Fresian and Jersey calves at 12 and 76 days of age. Anim. Prod. 12**:**493-501.

Jarrett, A. 1977. The Hair Follicle. Academic Press, London.

Kooistra, L.H., and O.J. Ginther. 1975. Effect of photoperiod on reproductive

activity and hair in mares. Am. J. Vet. Res. 36**:**1413-1419.
Levine, R.J., R.M. Moore, G.D. Maclaren, W.F. Barthel, and P.J. Landrigan. 1976. Occupational lead poisoning, animal deaths, and environmental contamination at a scrap smelter. Am. J. Public Health 66:548-552.
Lewis, L.D. 1995. Equine Clinical Nutrition: Feeding and Care. Williams and Wilkins, London.
Lloyd, D.H. 1993. Structure, function and microflora of the skin. Manual of Small Animal Dermatology. P. Harvey, R.G. Harvey, and I.S. Mason (Eds.). pp. 10-22. British Small Animal Veterinary Association, Cheltenham, UK..
Lowe, J.E., R.H. Foot, B.H. Baldwin, R.B. Hillman, and F.A. Kallfelz. 1987. Reproductive patterns in cyclic and pregnant thyroidectomized mares. J. Reprod. Fertil. Suppl. 35**:**281-288.
Lyne, A.G., and B.F. Short. 1965. Biology of the Skin and Hair Growth. Angus and Robertson, Sydney. McLaughlin, J.G., and J. Cullen. 1986. Clinical cases of chronic selenosis in horses. Irish Vet. J. 40**:**136-138.
Messenger, A.G. 1993. The control of hair growth: An overview. J. Invest. Dermatol. 101**:**4S-8S.
Mihajlovic, M. 1992. Selenium toxicty in domestic animals. Glas. Srp. Akad. Nauka. Med. 42**:**131-144.
Misir, R., R. Blair, and C.E. Doige. 1986. Development of a system for clinical evaluation of the biotin status of sows. Can. Vet. J. 27**:**6-12.
Muller, G.H. 1989. Small Animal Dermatology. W B Saunders Company, Philadelphia. Nakahara, Y. 1999. Hair analysis for abused and therapeutic drugs. J. Chromatogr. B. Biomed. Sci. Appl. 733:161-180.
Oxender, W.D., P.A. Noden, .and H.D. Hafs. 1977. Estrus, ovulation, and serum progesterone, estradiol, and LH concentrations in mares after an increased photoperiod during winter. Am. J. Vet. Res. 38**:**203-207.
Parker, F. 1981. Skin and hormones. In: Textbook of Endocrinology. R. H. Williams (Ed.). pp. 1080-1098.W B Saunders, Philadelphia.
Pilliner, S., and Z. Davies. 1996. Equine Science, Health and Performance. Blackwell Science, London.
Popot, M.A., S. Boyer, P. Maciejewski, P. Garcia, L. Dehennin, and Y. Bonnaire. 2000. Approaches to the detection of drugs in horse hair. Proc. 13th Internat. Conf. of Racing Analysts and Veterinarians, Cambridge, UK.
Potsch, L., G. Skopp, and M.R. Moeller. 1997. Biochemical approach on the conservation of drug molecules during hair fibre formation. Forensic Sci. Int. 84**:**25-35.
Randall, V.A., and F.J.G. Ebling. 1991. Seasonal changes in human hair growth. Br. J. Dermatol. 124:146-151.
Robbins, C.R. 1979. Chemical and Physical Behaviour of Human Hair. Van Nostrand Reinhold Co., New York.
Rook, A., R. Dawber. 1982. Diseases of the Hair and Scalp. Blackwell Scientific Publications, Oxford.

Ryder, M.L. 1976. Seasonal changes in the coat of the cat. Res. Vet. Sci. 21**:**280-283.

Samata, T. 1985. A biochemical study of keratin. I. Amino acid compositions of body hair and hoof of Equidae. J. Fac. General Educ. Azabu. Univ. 18**:**17-34.

Samata, T., and M. Matsuda. 1988. Studies on the amino acid compositions of the equine body hair and the hoof. Jpn. J. Vet. Sci. 50:333-340.

Scott, D.W. 1988. Large Animal Dermatology. W B Saunders, Philadelphia.

Sippel, W.L., J. Flowers, J. O'Farrell, W. Thomas, and J. Powers. 1964. Nutrition consultation in horses by aid of feed, blood and hair analysis. Proc. Amer. Assoc.Equine Pract. 10:139-152.

Smith, F.P., and M.S. Pomposini, M.S. 1981. Detection of phenobarbital in bloodstains, semen, seminal stains, saliva, saliva stains, perspiration stains and hair. J. Forensic Sci. 26**:**582-586

Smith, H., S. Forshufvud, and A. Wassen. 1962. Nature 194:725-726. Stenn, K.S., and R. Paus. 2001. Controls of hair follicle cycling. Physiol. Rev. 81**:**449-494.

Tagliaro, F., F.P. Smith, Z.D. Battisti, G. Manetto, and M. Marigo. 1997. Hair analysis, novel tool in forensic and biomedical sciences: New chromatographic and electrophoretic/electrokinetic analytical strategies. J. Chromatogr. B. 689**:**261-271.

Talukdar, A.H., M.L. Calhoun, and A.W. Stinson. 1972. Microscopic anatomy of the skin of the horse. Am. J. Vet. Res. 33:2365-2390.

Thompson, D.L., R. Hoffman, and C.L. DePew. 1997. Prolactin administration to seasonally anoestrous mares: Reproductive, metabolic and hair-shedding responses. J. Anim. Sci. 75:1092-1099.

Thornton, M.J., N.A. Hibberts, T. Street, B.R. Brinklow, A.S.I. Loundon, and A.V. Randall. 2001. Androgen receptors are only present in mesechyme-derived dermal papilla cells of red deer (*Cervus elaphus*) neck follicles when raised androgens induce a mane in the breeding season. J. Endocrinol. 168:401-408.

Tregear, R.T. 1965. Hair density, wind speed and heat loss in mammals. J. Appl. Physiol. 20**:**796-801.

Underwood, E.J. 1977. Trace Elements in Human and Animal Nutrition. Academic Press, New York.

Vale, M.M., and D.M. Wagoner. 1997. The Veterinary Encyclopedia for Horsemen. Equine Research Inc., Texas.

Valente, D., M. Cassini, M. Pigliapochi, and G. Vanzetti. 1981. Hair as the sample in assessing morphine and cocaine addiction. Clin. Chem. 27**:**1952-1953.

Ward, N.I., and J.M. Savage. 1994. Elemental status of grazing animals located adjacent to the London Orbital (M25) motorway. Sci. Total Environ. 146:185-189.

Wertz, P.W., and D.T. Downing. 1988. Integral lipids of human hair. Lipids 23:878-881.

Wesson, J.A., and O.J. Ginther. 1982. Influence of photoperiod on puberty in the female pony. J. Reprod. Fertil. Suppl. 32:269-274.

Whittem, T., C. Davis, G.D. Beresford, and T. Gourdie. 1998. Detection of morphine in mane hair of horses. Aust. Vet. J. 76:426-427.

Whittem, T., J. Foreman, and S. Wood. 2000. Disposition of cocaine in plasma and mane hair of horses after intravenous, buccal and rectal administration. Proc. 8th Internat. Congr. of the European Association of Veterinary Pharmacologists and Toxicologists, Jerusalem, Israel.

Wichert, B., T. Frank, and E. Kienzle. 2002. Zinc, copper and selenium status of horses in Bavaria. J. Nutr. 132:1776S-1777S.

Williams, D., P. Siock, and, K. Stenn. 1996. 13-cis-retinoic acid affects sheath-shaft interaction of equine hair follicles in vitro. J. Invest. Dermatol. 106**:**356-361.

Witte, S.T., L.A. Will, C.R. Olsen, J.A. Kinker, and P. Miller-Graber. 1993. Chronic selenosis in horses fed locally produced alfalfa hay. J. Am. Vet. Med. Assoc. 202:406-409.

Wysocki, A.A., and R. Klett. 1971. Hair as an indicator of the calcium and phosphorus status of ponies. J. Anim. Sci. 32**:**74-78.