PYRROLIZIDINE ALKALOID PLANTS, METABOLISM AND TOXICITY

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ABSTRACT/INTRODUCTION

More than 350 PAs have been identified in over 6,000 plants in the Boraginaceae, Compositae, and Leguminosae families (Table 1). About half of the identified PAs are toxic and several have been shown to be carcinogenic in rodents. PA-containing plants have worldwide distribution, and they probably are the most common poisonous plants affecting livestock, wildlife, and humans.

In many locations, PA-containing plants are introduced species that are considered invasive, noxious weeds. Both native and introduced PA-containing plants often infest open ranges and fields, replacing nutritious plants. Many are not palatable and livestock avoid eating them if other forages are available. However, as they invade fields or crops, plant parts or seeds can contaminate prepared feeds and grains which are then readily eaten by many animals. Human poisonings most often are a result of food contamination or when PA-containing plants are reused for medicinal purposes.

This is a review of current information on the diagnosis, pathogenesis, and molecular mechanisms of PA toxicity. Additional discussion includes current and future research objectives with an emphasis on the development of better diagnostics, pyrrole kinetics, and the effects of low dose PA exposure.

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PLANT CHARACTERISTICS AND DISTRIBUTION

PA-containing plants of three plant families found in many different environments and locations have caused animal and human health problems. Most PA-containing plants contain mixtures of alkaloids with varying concentrations and toxicities. In addition to the plant species that have been associated with animal and human poisoning (Table 1), there are numerous additional plants that contain toxic PAs, but have not been associated with poisoning. This suggests that in the future with changes in plant populations and distribution, alterations in livestock management, or as improved diagnostics identify currently unrecognized poisonings, additional plant species will need to be added to this list. The following section is a brief review of the common toxic PA-containing plants.

Senecio (Family - Compositae)

There are nearly 3000 Senecio species distributed throughout the world. Although many contain toxic PAs, just over 30 have been associated with livestock and human poisonings (Mattocks, 1986; Johnson et al., 1989). These species are morphologically variable with the common characteristics including the presence of a single whorl of bracts forming a cup under the head and seeds that are produced in disc and ray florets.

In the early 1800s, several diseases or syndromes were recognized that caused jaundice, liver cirrhosis, and hepatic encephalopathy. In Wales the disease was termed "stomach staggers." It was called "Pictou disease" in Nova Scotia, "Winton disease" in New Zealand, "Molteno" and "dunzieke" disease in South Africa, and "sirasyke" in Norway. Many similar unnamed syndromes were identified in other countries. Over the past 40 years, detailed field and experimental studies have positively identified the cause of these diseases as Senecio spp. intoxication. In the following paragraphs several Senecio species that are commonly associated with poisoning are briefly described.

Senecio jacobaea

Probably the most common and widely distributed species, S. jacobaea or tansy ragwort (Fig. 1), is a noxious weed that is a native of the British Isles (Ford et al., 1968; Thorpe and Ford, 1968; Giles, 1983). In the past century, it has been inadvertently introduced into Western Europe, South Africa, Australia, New Zealand, and North America. Tansy ragwort is a tall (0.5-1.5 m) erect plant that is unbranched except at the inflorescence. In the spring it forms a tall, flowering stalk with composite heads that have terminal, flat-topped clusters about 1 cm tall and individual 1 cm yellow rays. Each plant produces thousands of wind dispersed seeds. Leaves are variably sized up to 22 cm long and 10 cm wide with 2-3 deep pinnate divisions. It is commonly found invading well-drained pastures, forests, and wastelands.

As with most PA-containing plants, tansy is unpalatable and generally not eaten by livestock. Poisoning occurs when plants contaminate feeds, when grazing animals cannot easily differentiate the early rosette from grasses and clovers, or when no other forages are available. Senecio jacobaea contains 6 major alkaloids of which several have been shown to cross the placenta and to be secreted in milk (Johnson, 1976; Goeger et al., 1979; Johnson and Smart, 1983). Johnson (1979) reported that the chronic lethal dose of tansy ragwort in cattle is about 2.5 mg total PA/kg body weight/day when fed for 18 days. As the average PA concentration found in tansy ragwort is about 0.2% of the plant dry weight, a 300 kg cow would need to eat about 0.4 kg of dry plant or nearly 1.7 kg of fresh plant per day for 18 days to obtain a lethal dose. In the past such PA concentrations have been found as contaminants in feeds. Higher doses cause more severe hepatocellular necrosis with rapid onset of clinical disease. However, this is unlikely as such high PA doses would require large amounts of unpalatable plant that would not likely be ingested (Johnson et al., 1976).

Numerous additional studies describing clinical and post-mortem changes of S. jacobaea intoxication of various animal species
Table 1. Pyrrolizidine alkaloid containing Boraginaceae, Compositeae, and Leguminosae plants that have been associated with poisoning.

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<thead>
<tr>
<th>Family Compositae</th>
<th>Family Leguminosae</th>
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<tr>
<td>Senecio abyssinicus</td>
<td>Crotolela anagyroides</td>
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<td>S. abbyssinicus</td>
<td>C. assamicna</td>
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<th>Family Boraginaceae</th>
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<td>Amsinckia intermedia</td>
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<td>Cynoglossum officinale</td>
<td>Cattle, Horses [Knight 1984, Baker 1989]</td>
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<td>Heliotropium amplexicae</td>
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<td>H. dasycarpum</td>
<td>Sheep [Muratov 1973]</td>
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<td>H. lasiocarpum</td>
<td>Man [Cuileneo 1986, Chauvin 1994]</td>
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<td>H. ovolifolium</td>
<td>Sheep, Goats [Abu-Damir 1982]</td>
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<td>H. scoticus</td>
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<td>H. siporum</td>
<td>Rats [Schoental 1970]</td>
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<td>S. peregrinum</td>
<td>Poultry [Lee 1984]</td>
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<td>Trichodesma elenbergeri</td>
<td>Poultry [Shevchenko 1973, Wassel 1987]</td>
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have been published (see Cheeke and Pierson-Goeger, 1983; Craig et al., 1991, for reviews). Human poisonings have also been reported and there is a potential that people may be exposed to tansy PAs through contaminated milk or animal products (Cheeke, 1979). Accidental ingestion can occur since tansy ragwort also shares its common name, tansy, with *Tanacetum vulgare*, a commonly used herb.

**Senecio riddellii**

Around 1930 a disorder commonly called "walking disease" was identified in horses in the North American states of Nebraska, Colorado, and Wyoming. The name reflects the clinical presentation since affected animals often walked aimlessly in circles or along fences. This disease has since been proven to be caused by *S. riddellii* or Riddell's
grouse (Johnson et al., 1985b; Molyneux et al., 1991).

*Senecio riddellii* is a bright green, short (0.5 m) perennial shrub with glabrous stems and thread-like leaves. It has composite heads with flat-topped terminal clusters that have 6-8, short (1 cm) yellow rays. *Senecio riddellii* generally found in dry, desert areas, grows in the early spring and then dies back to the crown each fall. However, summer rains may allow sprouting of new growth on the woody stems. *Senecio riddellii* differs from the other PA-containing *Senecio* species in that it contains primarily a single alkaloid, riddelline (Johnson et al., 1985b). Interestingly, riddelline concentrations vary greatly with measured concentrations ranging between 0.2 and 18.0% (dry weight) in plants with similar phenotypes and locations (Johnson et al., 1989). *Senecio riddellii* is toxic to cattle at doses just over 15 mg PA/kg body weight/day when fed for 20 days (Johnson et al., 1985b). At 18% PA of the dry matter, 33 gm dry or 176 gm fresh *S. riddellii* per day would be toxic for a 300 kg cow. Although riddelline is less toxic than PAs from other *Senecio* species, the plant can contain more toxin, making the whole plant highly toxic. Recent experimental studies have also shown riddelline to be carcinogenic to rodents (Chan et al., 1994).

*Senecio douglasii* var. *longilobus*

Threadleaf or woolly grouse (S. douglasii var. longilobus) is a low-growing (30-60 cm) perennial, branched, woody shrub that is often found in arid areas. The leaves and stems are covered with a white woolly pubescence and leaves are up to 10 cm long with pinnate lobations. It has composite heads with numerous flat-topped terminal clusters with 13, yellow 0.5-0.8 cm long rays. Woolly groundsel is an opportunistic plant that becomes abundant on abused or degraded arid rangelands. Woolly groundsel has been reported to poison cattle in several southwestern states in North America (Mathews, 1933). It contains four alkaloids and the PA content varies from 0.63 to 2.02% of the dry weight of the plant (Johnson and Molyneux, 1984). Experimental feeding studies have shown that doses of 10-13 mg PA/kg body weight/day when fed for 15 days is lethal for cattle (Johnson and Molyneux, 1984). This suggests that at a PA concentration of 2%, doses of about 150 grams dry plant or 750 grams green plant for 15 days would be required to poison a 300 kg cow. That is about one-third the toxicity of *S. riddellii*.

*Senecio vulgaris*

Common groundsel is an erect, annual or biennial up to 45 cm tall. It has branched hollow stems with narrow pinnately lobed leaves and small, rayless, yellow flowers. It flowers much of the year and produces oblong, finely ribbed and haired achenes that have a fine white pappus that disperses the seeds. Common groundsel has been a tradition of usage for medicinal purposes and it has been recommended as a remedy for intestinal parasites, for disordered liver function, for delayed or irregular menstruation, as a laxative, or as a lotion for rough chapped hands (Mitich, 1995). Though livestock poisoning has been sporadic, *S. vulgaris* contaminated feeds have been reported to poison horses (Lecassard et al., 1986; Mendel et al., 1988). Subclinical poisonings have also been associated with altered reproductive function (Evans et al., 1987).

**Crotalaria (Family - Leguminosae)**

*Crotalaria* species have often been introduced into new areas to assist in improving soils as a result of their nitrogen-fixing capacity. As a result, residual plants often grow along fence rows and ditch banks where they spread to contaminate fields. Their seeds may be harvested with grains, thus contaminating feed and food. Several common *Crotalaria* species associated with livestock poisoning are listed in Table 1.

Most *Crotalaria* species are perennial branched legumes that grow from 20-50 cm to over a meter tall. They have simple, 3-5 cm long leaves with short pubescence on both
sides. They have winged petioles and the flowers (generally yellow) are about 0.5 cm long with a calyx longer than the corolla. They form leguminous pods about 2-3 cm long that are black when ripe. The 2-3 mm long seeds are generally kidney-shaped, brown, smooth, and they become detached and rattle in mature dry pods, resulting in the common name "rattle pod."

Several poisoning syndromes have been identified. A disease commonly called 'bottom disease' was recognized in the late 1800s to occur in Missouri river bottoms of the United States. Affected animals were slow, emaciated, weak, often seemed stuporous, with hepatic degeneration. It was uniformly fatal, as most animals died within a few weeks. After extensive field investigations and feeding trials, *C. sagittalis* was identified as the cause (Kingsbury, 1964). The disease became less frequent after the turn of century as horses (the most susceptible animal) were used less and feeds were less often contaminated. Recent poisonings include several in the northwest United States from *C. sagittalis* infested stubble fields (Kingsbury, 1964).

*Crotalaria* species have been used to increase soil nitrogen and organic material in the following years they spread and continue to grow in fields and on marginal lands. Consequently, *Crotalaria* seeds have contaminated grain, resulting in poisoning of both livestock and poultry (Williams and Molynex, 1987). In addition, since livestock are often fed grain screenings or grains that are regulated as unfit for human consumption, *Crotalaris*-contaminated grain can find its way into animal feed supplies. Although herbicides and improved processing to remove contaminants from feeds have reduced the incidence, the problem continues in many parts of the world (see Table 1 and associated references).

**Cynoglossum (Family - Boraginaceae)**

*Cynoglossum officinale* or hound's tongue is an annual or biennial plant forming a rosette the first year. It dies back in the winter and regrows from a taproot to form a flowering shoot (Fig. 2). The leaves are 8-20 cm long, gray-green, and softly hairy on both sides with smooth entire margins. The flowering stalk is 40-90 cm tall with terminal cymes containing up to 35 flowers. The flower corolla is 5-6 cm long, reddish purple with five lobes fused at the lower part into a cylinder. There are five stamens in the upper part of the cylinder. Each flower forms four nutlets that are 6-8 mm long, hook or glochidia covered (allowing animal dispersal), that turn brown when ripe.

Hound's tongue is a native of Europe that has been introduced into North America. As a noxious weed, hound's tongue often invades pastures, rangelands, and some cultivated fields. It is generally unpalatable to livestock, but cattle and horses have been poisoned when fed contaminated hay (Knight et al., 1984; Baker et al., 1989; Stegelmeier et al., 1996). Hound's tongue contains four PAs with heliosupine being the most abundant and toxic (Pfister et al., 1992). PA concentrations were highest in immature plants and ranged from 0.5 to 2.2%. About 10-15 mg PA/kg bw/day for 14 days was lethal for cattle and horses. This suggests that a lethal dose for a 300 kg cow would be 136 grams dry plant or 680 grams green plant per day for 14 days (Baker et al., 1991; Stegelmeier et al., 1996).

**Heliotropium and Echium (Family - Boraginaceae)**

Both *Echium* and *Heliotropium* species have intermittently poisoned Australian livestock. Though their toxicity has been experimentally proven, their toxicity in the field is still debated (Seaman and Dixon, 1989; Seaman et al., 1989). Perhaps this is best illustrated by the common name of *E. plantagineum*. In most areas *E. plantagineum* is called Patterson's Curse, as it is a noxious weed that replaces nutritious forages and poisons livestock. In other areas the plant is looked at quite differently, as it is called Salvation Jane. In these locations there are often droughts in which there is little available vegetation and *Echium* is often the only feed available. Sheep are relatively resistant to *Echium* toxicity and they experience little
detrimental effect from eating large quantities of dried plant (Culvenor et al., 1984). Other species, especially horses, are more susceptible to toxicity, and though it is not very palatable, they are occasionally poisoned (Seaman, 1978; Gisecke, 1986). *Echium* toxicity, as often seen with other hepatotoxins, disrupts normal liver metabolism, and several studies report associations of both *Echium* and *Heliotrope* poisoning with altered copper and molybdenum metabolism and subsequent copper intoxication (Seaman, 1985).

*Echium* species are hardy annual or biennial plants that, in the case of Patterson’s curse, were introduced from Europe. Patterson’s curse is from 30-60 cm high with erect, leafy, hairy stems. The leaves are ovate to lanceolate, green, and about 15 cm long with a
prominent midvein. The flowers are funnel-shaped with terminal cymes that have pink buds that later turn blue or white. It tends to grow in disturbed areas such as along roads and fences where it smothers other useful plants. Medicinally it has been used as a cure for snakebites and melancholy (Peterson and Jago, 1984; Giesecke, 1986; Seaman et al., 1989).

*Heliotropium* poisoning, especially *H. europaeum* poisoning, is reported to affect considerable numbers of livestock, particularly sheep, in Australia (McLennan et al., 1972; Jones et al., 1981; Peterson et al., 1992). Though it is found in the southern United States, it rarely poisons livestock there. It is an annual from the Mediterranean region that is often called common heliotrope. It is an erect plant growing up to about 30 cm with gray-green, oval leaves that are arranged alternately on thick stalks. Leaves and stems are covered in short, coarse, white hairs and have prominent veins. The flowers are small (less than 5 mm in diameter) and white with a yellow throat. Heliotrope seeds consist of a group of four single-seeded nutlets per flower, each 2 mm long. The outer surface is rough with a smooth, concave inner surface.

Though the use of broadleaf herbicides and improved management has reduced the incidence of livestock poisoning, PA contamination of honey and wildlife intoxications have renewed interest in *Echium* and *Heliotropium* species. In addition to the *Echium* and *Heliotropium* species discussed, several others have also been proven to be toxic. See Table 1 and the associated references for additional descriptions and details of these problems.

**Other PA-Containing Boraginaceae Plants**

*Amsinckia intermedia*, commonly called tarweed or fiddleneck, is an annual weed that grows in waste areas and fields. *Amsinckia* is a prolific, seed producing, erect annual that grows to nearly 1 meter. It is covered with white bristly hairs with lanceolate, alternate leaves. The flowers form on one side of a raceme that uncoils once the flowers ripen (fiddleneck). The fruit separate when they mature into two or four black, 2-3 mm nutlets. *Amsinckia* is not highly toxic, but it has been reported to cause "walking disease" in horses and "hard liver disease" in cattle and swine (McCulloch, 1940, 1942; Woolsey et al., 1952; Kingsbury, 1964).

*Symphytum officinale* or comfrey has been used as both forage and an herb. It contains several different PAs which cause disease in both experimental animals and humans (Brauchli et al., 1982; Furmanowa et al., 1983). Low doses of comfrey have been shown to produce hepatic neoplasms in rodents (Hirono et al., 1978). Although several countries have banned or restricted its sale, comfrey continues to be used in many herbal preparations.

Several other plants including members of the *Borago* and *Trichodesma* genera also contain small amounts of PAs (Table 1). Some of these plants are commonly used as medicinal plants and herbs. Unfortunately, there is little information concerning the effects of low dose PA exposure associated with these plants. More information is needed to determine the effect of such exposures and to determine if they alter normal physiologic processes including immunologic function, aging, and healing.

**PYRROLIZIDINE ALKALOID CHARACTERISTICS AND STRUCTURE**

The plant-derived hepatotoxic pyrrolizidine alkaloids (PAs) are a diverse class of alkaloids with considerable structural differences. However, all with demonstrated hepatotoxicity contain a 1,2 unsaturated necine base moiety which is generally esterified to one or more necic acids (Fig. 3). The PAs occur naturally as both the free base and N-oxide forms. However, in cattle at least, the N-oxides have been shown to be as toxic as the free bases (Molyneux et al., 1991). This suggests that the total alkaloid content (free bases and N-oxide combined) should be considered when estimating the risk from any particular plant. Toxicity of the PAs increases if both hydroxyls on the necine base are esterified, if the necic acids have specific
branched chains or centers of unsaturation, or if the necic acid is bifunctional, forming a macrocyclic diester ring (Fig. 3).

**METABOLISM**

Pyrrolizidine alkaloids are not toxic per se, but require bioactivation to the toxic dehydro-pyrrolizidine alkaloids (the so-called "pyroles") (Step 1, Fig. 3). This bioactivation occurs primarily in the liver by the action of several different mixed function oxidases (Mattocks, 1986). As with many toxins, metabolism includes mechanisms to both activate and detoxify PAs. The non-toxic metabolites are quickly excreted. Toxic species either damage the liver or they may be transported to other tissues. With appropriate leaving groups on carbons 7 and 9 of the dehydroalkaloids, these pyrrolic esters are potent electrophiles which undergo nucleophilic substitution reactions involving displacement of the necic acid moiety by cellular nucleophiles. Some information exists regarding the enzymology of PA oxidation. In rats, an important cytochrome P-450 (CYP), specifically the CYP 3A isoenzyme, has been shown to be responsible for production of the reactive pyrrolic intermediate of senecionine. However, another isoenzyme, CYP 2C11, appears to have a high activity toward N-oxidation (Williams et al., 1989a). Flavin-containing monooxygenases (FMO) isolated from pig liver also have been shown to N-oxidize senecionine (Williams et al., 1989b).

Pyrrolic intermediates are bifunctional electrophiles possessing carbon-centered electrophiles at the 7 and 9 position. These can cross-link (i.e., react at two sites) with two cellular nucleophiles, such as DNA, proteins, amino acids, and glutathione (Woo et al., 1993). In cultured bovine kidney cells, activated pyrroles cross-link and protein in a roughly equivalent proportion (Hincks et al., 1991).

There is also marked structure-activity with respect to cross-linking potency. For example, a,b-unsaturated macrocyclic diester PAs (such as seneciphylline, riddelline, retrorsine, senecionine) are more potent DNA cross-linkers, inhibitors of colony formation and inducers of megalocytosis than the a,b-saturated macrocyclic diester PA monocrotaline, the simple necine base (retronecine) or open diester (heliosupine) in cultured cells. The simple necine base retronecine is the least potent of these PAs (Hincks et al., 1991; Kim et al., 1993, 1995). These results indicate that PA-induced DNA bioactivity in cells is closely related to structural features.

Cellular defense mechanisms including soluble nucleophiles, such as glutathione, react with such alkylating pyroles and the resulting adducts are rapidly excreted (Yan and Huxtable, 1995a). Other pyroles may polymerize, becoming harmlessly sequestered, or excreted (Step 6). However, many react with cellular nucleophilic compounds including proteins and nucleic acids (Step 7). Depending on the extent, as well as the importance or location of the damage, these adducts are responsible for the cytotoxic and antimitototic effects of poisoning. Some pyrrole-tissue adducts may persist for months or years and little is known about their ultimate fate. The progressive nature of PA-induced hepatic toxicity suggests pyrrolic adducts are recycled, reacting with new nucleophiles and inciting further cellular damage (Step 8). PAs or their pyrrole metabolites may also be hydrolyzed, producing alcoholic dehydronesines (Step 2). These may be dehydrogenated (Step 4), but the resulting pyrrolic metabolites are less reactive, less toxic, more water soluble, and more easily excreted than the dehydro-PAs. Pyrrolizidine alkaloids and activated pyrroles may be further oxidized forming N-oxides (Step 3). These are excreted, but may be reduced to the basic alkaloid in some tissues or in the intestinal tract to be reabsorbed and metabolized again (Mattocks, 1986).

Despite similar structures, acute PA toxicity is highly variable. Toxicity appears to be PA or pyrrole specific. For example, Huxtable and co-workers (1995) showed that liver preparations infused with different PAs have different metabolism rates with production of specific patterns of PA metabolites. Using rat liver preparations, senechylamine and retrorsine increased bile production with
secretion the conjugate, 7-glutathionyl-6,7-dehydron-1-hydroxymethyl-5H-pyrrolizidine. The excretion rate for retorsine as its glutathione conjugate was over 10 times faster than for trichodesmine. However, trichodesmine-treated livers released large amounts of dehydroalkaloid (pyrrole) into the perfusate. Tissue-bound pyrrole adducts higher than any of the other PAs were found in livers perfused with senechalyline and retorsine (Yan and Huxtable, 1995b; Yan et al., 1995). These observations help explain the tissue
specificity of some PAs for the liver or for extrahepatic organs. More reactive metabolites impact the site of formation, i.e., the liver. Thus senecephylline and retorsine are primarily hepatotoxins while structurally similar, trichodermine and monocrotaline, cause few hepatic changes with extensive extrahepatic lesions.

As most pyrroles are produced in the liver, PAs are generally potent hepatotoxins. Highly reactive electrophiles, pyrroles are short-lived as they quickly bind and damage adjacent hepatic molecules. Some PAs or their metabolites are more stable and they may circulate and damage extrahepatic tissues. Recent studies suggest that activated pyrroles may be transferred to extrahepatic tissues (possibly as temporary adducts of soluble proteins). It has been shown that some PAs are circulated unchanged to other tissues where they are activated by local tissue mixed function oxidases. For example, pulmonary toxicity is partly a result of pyrrole production within pulmonary endothelial cells and type II pneumocytes (Huxtable, 1990). Extrahepatic damage in most situations appears to be associated with PA and pyrrole solubility and stability (Van Zwanenberg, 1973).

Pyrrolizidine alkaloid toxicokinetics are alkaloid specific. Hepatotoxic PAs such as retorsine develop high liver pyrrole concentrations that peak about two hours after a single PA dose. Hepatic pyrrole concentrations fall slowly over the next 24 hours, but residues can be detected days later. Non-hepatotoxic PAs such as rosmarinine have lower hepatic pyrrole production that is removed from the liver much more rapidly (see Mattocks, 1986, pp. 158-190, for review).

**ANIMAL SUSCEPTIBILITY**

Toxicity is influenced by many factors including species, age, sex, as well as other temporary factors such as biochemical, physiologic, and nutritional status. Different species have vastly different susceptibilities to PAs. For example, the toxic doses of some plants were 20 times higher for sheep than those that killed cattle. For this reason sheep and goats have been used to graze pastures that are dangerous to horses and cattle (Hooper, 1978). Horses appear to be about as sensitive to PA toxicity as cattle, but pigs are reported to be the most sensitive animal species. The reported toxicity indexes are: pigs = 1; chickens = 5; cattle and horses = 14; rats = 50; mice = 150; sheep and goats = 200 (Hooper, 1978).

Transfaunal studies using rumen microbes, *in vitro* studies of rumen cultures incubated with PAs, and animal studies using direct infusion of purified PAs in the portal circulation, all support the hypothesis that at least a portion of species susceptibility to PA toxicity is due to rumen metabolism (Craig et al., 1992; Wachenheim et al., 1992; Craig and Blythe, 1994). On the other hand, there are also studies that suggest species susceptibilities are more closely related to differing liver metabolism. Different species have different rates of both pyrrole activation and detoxification (Peterson and Jago, 1984; Winter et al., 1988). Additionally, species differences have been found in the amounts and rates of urinary excretion of N-oxide metabolites, suggesting differing abilities to excrete these toxins (Chu and Segall, 1991). This suggests that species susceptibility is a complex interaction involving alkaloid and animal specific differences in both pre-absorption and post-absorption metabolism. It also suggests that toxicity information from one species may be of little use to predict toxicity in other species. For example, guinea pigs are highly resistant to the toxicity of most PAs, but they are very susceptible to jacobine. Chung and Buhler (1995) suggested that this difference is a result of the guinea pig's unique ability to quickly produce jacobine-related hepatotoxic metabolites and relatively slow hydrolysis of jacobine esters and the slow oxidation to form the N-oxide. Other species differences are also likely to cause unique differences in PA metabolism.

Age and gender play a large role in determining an animal's response to PAs. Young animals are generally more susceptible to poisoning than aged adults (Chauvin et al., 1994). Neonatal and nursing animals have
been reported to develop fatal hepatic disease, while their lactating mothers were unaffected (Stillman et al., 1977; Roulet et al., 1988; Small et al., 1993; Sperl et al., 1995). Gender differences are seen as PA toxicity in male rats is much higher than that seen in females (Williams et al., 1989). These differences have been linked to metabolic rates (Chauvin et al., 1994). It has been postulated that toxicity is linked to both the ability of the liver to synthesize pyroles as well as the proliferation, growth, or metabolic rates of the liver.

The physiologic state of the animal also contributes to toxicity. Nutritional status is important especially relating to nutrients that rely heavily on hepatic metabolism such as copper and molybdenum (Peterson et al., 1992; Seaman, 1985). As mentioned previously, pyrolic esters may alkylate nucleophilic scavengers such as glutathione with consequent excretion of the glutathione adduct. Therefore, animals with increased hepatic glutathione might be expected to be more resistant to PA toxicity (Petry and Sipes, 1987; Yan and Huxtable, 1995a, 1996). Hepatic bioactivation of PAs can be promoted or inhibited by treatments that up-regulate, enhance, or otherwise alter hepatic microsomal activity (phenobarbital, chloramphenicol, etc.) (Petry and Sipes, 1987; Chung et al., 1995).

Plant and animal factors also contribute to toxicity. Palatability, the amount and rate of plant that animals will eat, varies with season, location, weather, and the availability of other forages. PA concentration in plants varies with the environment, plant phenotype, and site. Usually the plants are most toxic in the early bud stage when beginning to flower. Nevertheless, there are large variations in PA concentrations from year to year and from site to site (Johnson et al., 1985a). This makes it difficult to predict when a particular group of plants will contain toxic PA concentrations. More work is needed to understand the cause of these differences and to better detect or predict PA concentrations in plant populations. Current recommendations are that one should recognize these potentially toxic plants, know the susceptible animal species, and take necessary precautions to insure that they are not exposed (Mattocks, 1986).

**PATHOGENESIS**

As mentioned earlier, activated PAs or pyroles are potent bifunctional alkylating agents that quickly bind with available cellular nucleophiles. Much work has been done to identify specific proteins or nucleic acids targets with which pyroles are likely to form adducts. Many pyroles have been shown to bind with genomic DNA and nuclear proteins (Mattocks, 1986). The formation of DNA-protein or DNA-DNA interstrand cross-links correlates with many of the toxic sequelae of PAs (Petry et al., 1984, 1986; Candrian et al., 1985; Reed et al., 1988; Hincks et al., 1991).

Functionally, the adducts and cross-linked proteins and nucleic acids are likely to inhibit the normal genomic unfolding, RNA transcription and translation, DNA synthesis, and chromosomal mitotic movement. It has been hypothesized that these changes are responsible for the antimitotic effect and the consequent large hepatocytes (megakaryocytes) associated with PA intoxication (Hincks and Coulombe, 1989; Kim et al., 1993). This is supported by earlier studies that found that pyroles inhibit cell mitosis in late S or early G2 phase (Samuel and Jago, 1975). Cytosolic proteins and nucleic acids are also common targets and pyrole adducts are commonly formed with thiol groups of many proteins (Huxtable and Wild, 1994). Such protein damage will likely result in loss of function, decreased energy production, and loss of homeostasis.

Recently, actin was identified as a major protein cross-linked by dehydromonocrotaline and dehydrocrotaline in cultured bovine and human cells and isolated nuclei (Coulombe et al., 1998). Because it was previously shown that the megakaryocytic and antimitotic effects of PAs coincide with cross-linking potency (Kim et al, 1993), it is plausible that the antimitotic action of pyrolic PAs in vitro and in vivo may be explained, at least in part, by their ability to cross-link DNA with actin.

Cellular indications of PA intoxication
are first seen as hepatocyte swelling that is dose dependent. With continuing damage, cellular degeneration continues with ultimate loss of cellular homeostasis and necrosis of cell death. The lesions of PA intoxication which are dose dependent have been histologically characterized as acute and chronic changes. High PA doses ingested quickly generally cause changes recognized as acute intoxication. Such animals develop panlobular hepatocellular damage characterized by acute extensive necrosis with hemorrhage and minimal inflammation (Fig. 4). These animals show signs of acute liver failure including anorexia, depression, icterus, visceral edema, and ascites. Such cases can be diagnostic challenges as other toxic, viral, and immunologic diseases can cause similar hepatic necrosis. Fortunately, these animals have high concentrations of tissue-bound pyroles that can be extracted and detected chemically.

Lower PA doses over longer duration or chronic intoxication result in less severe lesions that include focal hepatocyte necrosis (piecemeal necrosis), peribiliary fibrosis, and bile duct proliferation. With time, damaged hepatocytes develop into large megalocytes (Fig. 5). Chronically poisoned animals often show no clinical signs and their serum biochemistries may be normal for several months or even years after PA ingestion. However, hepatocellular damage continues, resulting in increased hepatocyte death with subsequent inflammation, fibrosis, and ultimately cirrhosis. With loss of hepatic function, poisoned animals often do poorly and when subjected to normal stresses such as pregnancy or lactation, they develop clinical liver failure. Chronic PA intoxication may present with clinical signs of photosensitivity, icterus, or increased susceptibility to other hepatic diseases such as lipidosis or ketosis. As disease often develops months after PA exposure, it is often difficult to definitively identify the PA-containing plant or feed contaminant. Additionally, initial kinetic studies suggest that chronically poisoned animals often have low concentrations of tissue-bound pyroles (unpublished results). This makes it difficult to confirm intoxication using chemical detection techniques. Of course, some animals exposed to low PA concentrations may never develop liver disease. More information is needed to identify these ‘safe’ exposure limits.

The progressive nature of chronic PA intoxication suggests that low, chronic PA exposure has cumulative effects. Currently, little is known about what doses or durations will ultimately be damaging. Less is known if such subclinically intoxicated animals have impaired growth or productivity. More work is needed to better define this progression, to identify markers to predict how animals will progress after exposure, and to determine the fate and possible clearance of tissue-bound pyroles in animals that recover. Although various treatments and diet supplements have been suggested, none have proven to be effective in livestock (Johnson, 1979). Generally, poisoned animals that show clinical signs rarely recover.

**HUMAN POISONING**

The largest human poisonings occur when plant parts or seeds are ingested accidentally as a result of food or grain contamination. Such epidemic PA poisonings have occurred in Russia (Tadjikistan, Uzbekistan), Japan, Nigeria, Afghanistan, India, Sri Lanka, South Africa, Iraq, and the Caribbean (Mattocks, 1986; Huxtable, 1989; Yan et al., 1995). This is most often a result of PA-containing plants growing in wheat, millet, or similar cereal crops. The harvested grain and weed seeds are then milled into flour, which is made into bread. The resulting disease, often called ‘bread poisoning,’ has a higher incidence in young children with a clinical course that can last between 14 days to over several years. The largest reported incident occurred in Afghanistan in 1974 when over 35,000 people in nearly 100 villages were affected and many died (Tandon et al., 1978). Currently, epidemic poisonings are less common since herbicide use and grain inspection have reduced field infestations and improved grain quality. As mentioned previously, although contaminating seeds can be removed from cereals by screening, PA residues can often be detected in the cleaned
Figure 4. Photomicrograph of liver from a horse with acute pyrrolizidine alkaloid intoxication. Note the extensive necrosis (n) and hemorrhage (star) adjacent to biliary epithelium (b). Fibrosis, biliary proliferation, and megalocytosis are generally not seen in acute poisonings (H&E, Bar = 50 μ).

Grain. Despite the fact that the effect of such low dose exposure is unknown, several nations are moving towards establishing regulations to control contamination.

Pyrrolizidine alkaloids have been identified in other foods including milk and honey (Dickinson et al., 1976; Goeger et al., 1979; Segall and Krick, 1979). As goats are especially resistant to PA toxicity, there is a possibility that such animals ingesting PAs could excrete enough toxin in the milk to be harmful to susceptible animals (Molyneux and James, 1990). Honey made by bees collecting nectar from PA-containing plants also have low PA concentrations (0.06 mg/kg) (Culvenor et al., 1981; Crews et al., 1997). Though these
concentrations are below those thought to be toxic, the fact that PA are often carcinogenic and genotoxic suggests that information is needed to determine the risks of low PA doses of long duration (Molyneux and James, 1990). If pyrrole-induced damage is cumulative, such low doses could be a public health concern. The second frequent mechanism of human poisoning is when PA-containing plants are used for medicinal or herbal purposes. In the past few years, there has been an explosion of interest in the medicinal use of plants and herbal medicine. Through deliberate use of PA-containing plants or contamination of
herbal preparations, numerous people have been poisoned throughout the world. Especially at risk are young children and babies, and there are several reports of infants developing veno-occlusive disease when they were treated with herbal teas or when their mothers used such an herbal preparation during pregnancy (Stillman et al., 1977; Roulet et al., 1988; Sperl et al., 1995). As a result, the German Federal Health Department has limited the use of over 600 herbal remedies. These include comfrey, borage, purging buckthorn, colt’s foot, and groundsel plants that all contain unacceptably high PA concentrations. The daily exposure has been restricted to no more than 0.1 μg PA per day for no more than 6 weeks in a year (Anon., 1992). Similar regulation has been proposed in several other countries.

PA-induced histologic lesions in man are similar to those previously described for other animals with the exception of humans are more likely to develop veno-occlusive disease. Veno-occlusive disease characteristically involves occlusion of the smaller branches of the hepatic vein, due to endothelial proliferation and medial hypertrophy with perivascular fibrosis (Allen et al., 1969, for review). Although damage to other organs including the lung (pulmonary hypertension and cor pulmonale) has been reported in human PA poisonings, these changes are infrequent (Huxtable, 1989). PAs have not been associated with human carcinogenesis; however, they are carcinogenic rodents. These carcinogenic doses used in rodents are comparable to concentrations received by people using PA-containing plants for medicinal purposes. More work is needed to better define the carcinogenic effects of PAs and determine the risks, especially for low dose exposures over extended periods of time.

**DIAGNOSIS**

As many signs of poisoning do not develop until months after exposure, it is often difficult to document exposure to PA-containing plants. As a result, most diagnoses are currently made using histologic changes alone. Many of the histologic lesions of poisoning (hepatic necrosis, fibrosis, biliary proliferation, and megalocytosis) are nonspecific changes that can be initiated by a variety of toxic and infectious agents. The ubiquitous nature of PA-containing plants suggests that PA intoxication is underdiagnosed and that therefore the actual incidence is much larger than current estimates. Chemical methods using both spectrophotometry or gas chromatography/mass spectrometry have been developed to detect tissue-bound pyrroles (PA-metabolites) (Mattocks and Jukes, 1990; Stegelmeier et al., 1996; Schoch et al., submitted). Although these have proven to be useful in identifying exposed animals, they lack sensitivity and they are not quantitative. Recent studies found little correlation between the PA dose and residual tissue-bound pyrroles (Schoch et al., submitted). Hopefully, improved sensitive diagnostics including ELISA-based immunodiagnostics will provide better information on pyrrole kinetics, possible pyrrole recycling, or the cumulative effects of poisoning.

**FUTURE DIRECTION**

Current PA research objectives are to develop better diagnostic techniques to identify poisoned animals, monitor feeds or food for contamination, and better study pyrrole kinetics in exposed animals. ELISAs have been developed for specific PAs (Bober et al., 1989, 1991; Roseman et al., 1992, 1996; Roeder and Pfleuger, 1995). However, none have been developed with the specificity and sensitivity to be used for screening feeds and food or identifying PA in animal tissues. This work needs to be initiated. Additional information is also needed on the effects of low PA doses with prolonged exposure. As neonates appear to be especially sensitive to poisoning, models using young animals would provide the best estimate of tolerable exposure limits. Additional work is needed in developing models to better identify risks, formulate regulations, and better prognose the outcome for exposed animals.
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