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Title: Factors Affecting the Toxicity of Senecio jacobea to Laboratory Animals

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Senecio jacobaea, a weed prolific in the Pacific Northwest, causes considerable economic loss through livestock death. Commonly tansy ragwort, the plant is toxic to cattle and horses, while sheep seem to be resistant.

Seven laboratory animal species were evaluated as to their response to the toxic effects of the six pyrrolizidine alkaloids in tansy ragwort. Gerbils, guinea pigs, hamsters, Japanese quail, mice, rabbits and rats were fed ten percent and/or thirty percent ground tansy ragwort in their diets. Lethal tansy ragwort intake as a percent of initial body weight was 3639% in the gerbil, while only 6% in the rat. In contrast to published studies in which guinea pigs were quite resistant to the pyrrolizidine alkaloid monocrotaline, guinea pigs died after consuming only 119% of initial body weight as tansy ragwort.

Response to injection of alkaloids extracted from the plant was

also noted. Monocrotaline, jacobine (one of six alkaloids in tansy ragwort), and a composite of all six tansy ragwort alkaloids were tested. Guinea pigs were resistant to 500 mg/kg of monocrotaline, but died three days after injection of 100 mg/kg of jacobine or composite tansy ragwort alkaloid. Rats and gerbils were resistant to 150 mg/kg of monocrotaline, but died two days after injection of composite tansy ragwort alkaloid. Rabbits, although resistant to ingested tansy ragwort, died 24 hours after injection of 120 mg/kg of the composite alkaloid preparation. This differing response between ingested and injected pyrrolizidine alkaloids is also observed in Japanese quail. The LD_{50} for composite tansy ragwort alkaloid in quail was 115 mg/kg.

Gerbils and Japanese quail appeared to be most resistant to pyrrolizidine alkaloid poisoning, while rats were most susceptible. These species may be valuable as laboratory animal models for livestock.

Several theories on detoxifying tansy ragwort were explored. The most promising results were obtained by soaking tansy ragwort in alkali (4N NaOH) for 48 hours. After testing a variety of pH's, pH 7.5 appeared to be optimum for allowing maximum degradation of pyrrolizidine alkaloids without causing sodium-chloride imbalances in the body. Extent of detoxification was measured by the length of survival times of rats consuming diets containing treated tansy ragwort. Survival times for rats consuming fifteen percent tansy ragwort in their diets were increased from 50.9 days with untreated tansy ragwort to 151.7 days for those receiving tansy ragwort soaked at pH 7.5. Alkali treatment causes an irreversible change in the alkaloids as shown by similar results ob-

tained with alkaline soaked tansy ragwort that had been re-acidified to pH 4.0. These results suggest that further studies should involve incubation of tansy ragwort with rumen fluid buffered at different pH's with the long-term objective of developing a feed additive for altering rumen pH to increase resistance of cattle to tansy ragwort toxicity.

Other treatments were also evaluated as to their effect of reducing the toxicity of pyrrolizidine alkaloids to rats. Soaking in autoclaved cattle rumen fluid improved survival time from 83.0 days with untreated tansy ragwort to 123.7 days. Sulfur containing amino acids, mineral oil, Tris buffer and ion exchange resins were tested as dietary additives. None had any effect on improving survival time.

FACTORS AFFECTING THE TOXICITY OF SENECIO JACOBEA TO LABORATORY ANIMALS

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FACTORS AFFECTING THE TOXICITY OF SENECIO JACOBEA TO LABORATORY ANIMALS

I. INTRODUCTION

Tansy ragwort* (Senecio jacobea) is a yellow flowered weed, toxic to most livestock. The plant is widely distributed, occurring on five continents. In Oregon, it has been observed in all but five of the 36 counties (Figure 1). Infestation of pasture and cropland is increasing. Annual economic losses for Oregon have been estimated, ranging upwards to $10 million (Isaacson, 1976, personal communication). This figure includes cattle and horses poisoned, forage and seed crops contaminated, and funds spent on control of the plant.

Many control methods have been devised, ranging from pulling the plant out by the roots, to strong chemical weed killers. Recently, two insects, the cinnabar moth and the flea beetle have been employed as biological controls. Sheep are also being used to graze tansy ragwort-infested pastures due to their apparent resistance to the toxicity. However, the proliferation of the plant is too rapid for any of these current methods to remedy the problem. The toxicity of tansy ragwort is due to the pyrrolizidine alkaloids it contains. These alkaloids are present in several plant families. The Compositae family, of which tansy ragwort is a member, also contains other poisonous pasture weeds

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* Tansy ragwort (Senecio jacobea), although referred to in this text as "tansy" is not to be confused with Tansy (Tanacetum vulgare L.).

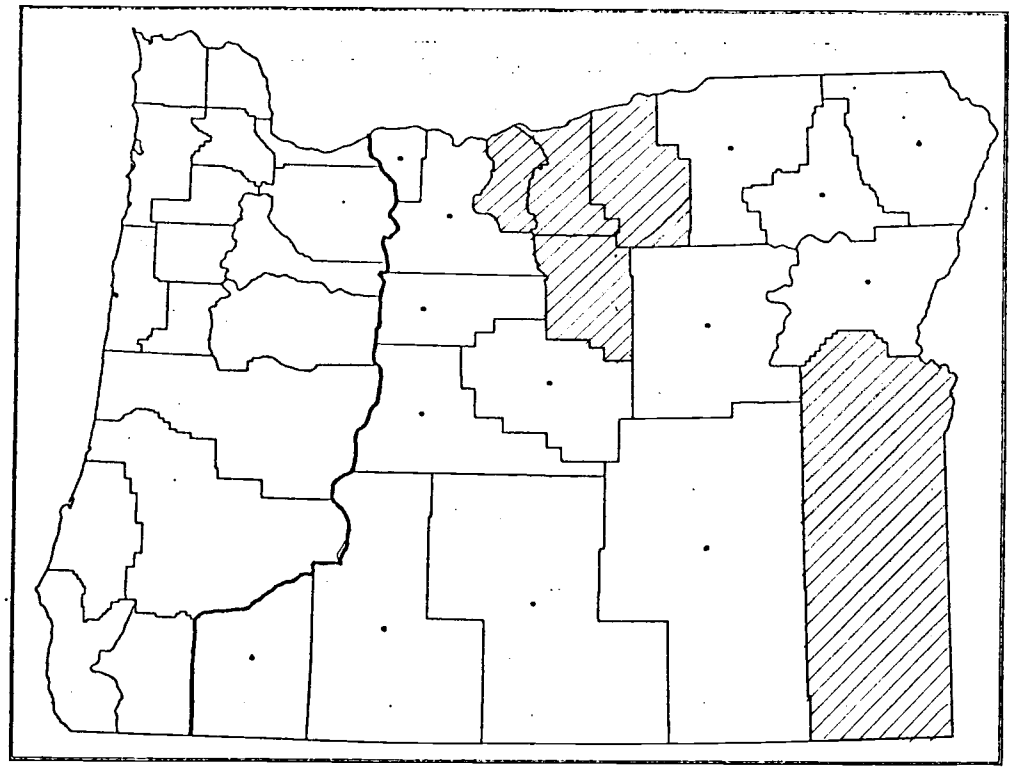


Figure 1. Distribution of tansy ragwort in Oregon.

Left of heavy line -- widespread in all counties.
Right of heavy line -- plants discovered in counties with dot.
Shaded counties -- no plants discovered.

(Isaacson, 1976, personal communication)

such as Senecio vulgaris (groundsel).

This present research approaches the tansy ragwort problem from two angles. First, identification of susceptible and resistant species for use as laboratory animal models could aid in the determination of the biochemical mechanism of toxicity. Second, the effect of dietary additives on reducing the potency of tansy ragwort to rats was investigated.

II. LITERATURE REVIEW

Historical Aspects

Tansy ragwort first arrived in New Zealand by ship from Europe around 1850, and the plant spread rapidly. In the 1880's, many cattle and horses succumbed to liver disease (Winton disease). One theory implicated tansy ragwort consumption as the cause (Park, 1896). Although some farmers disagreed with this view, many made efforts to control the weed. Sheep, apparently safe from the disease, were allowed to graze the tansy infested pastures. Some felt that breaking up the land for three or four years kept the plant from growing. Fines were imposed for allowing tansy ragwort to grow on one's property.

Another widespread outbreak of liver disease resulting in livestock death occurred in 1902. The winter had been hard, and aside from tansy ragwort, few plants had survived. Cattle had no choice but to eat tansy. Pursuing the theory that this weed was responsible for the deaths, Gilruth (1902) fed two six-month old calves a diet containing 60% dried tansy ragwort ad lib for 18 days. Each animal consumed 108 pounds of tansy. Twenty-eight days after the experiment was begun, one of the calves died; the other died two days later. Examination of the livers revealed abnormal amounts of fibrous tissue, congested capillaries, hepatic giant cells, and a thickened hepatic capsule.

These findings promoted further investigation. To determine whether the plant was still toxic after cutting and drying, Gilruth (1903) made a diet of withered tansy fodder. Two cows, one receiving

one pound of tansy per day, the other two pounds of tansy per day, ate satisfactorily. A horse getting .75 pound of tansy per day showed extreme dislike for the feed. After three months of experimental feeding, the cow which had been on the two pounds per day diet was slaughtered and autopsied. There were no abnormalities, gross nor histological. Based on these findings, Gilruth assumed that the dried plant had lost its toxicity.

Gilruth had observed sheep eating tansy for considerable lengths of time with no apparent dislike. It was rumored that prolonged periods of consumption would result in death. A flock of sheep was confined to land densely covered with tansy (Gilruth, 1904). No suspicious deaths occurred during the first year, although several complaints were received from butchers about excessively fat and yellowed carcasses. The second year brought on considerable mortality. Autopsy showed yellowed livers in which the lobules were very distinct.

Other early investigations by Gilruth included determining if the tansy ragwort disease was contagious or could be contracted from contact with stables having previously housed animals dying from it, if inadequate feeding increased susceptibility, and if sun cured ragwort fed with bran caused death in cattle (Gilruth, 1905).

In the same decade that tansy ragwort appeared in New Zealand, the plant was brought by Scottish ships to Nova Scotia (Cushny, 1911). Breeding of mares became nearly impossible because of the development of Pictou disease (Winton disease) in horses consuming ragwort for two years. However, a government study conducted in 1882 failed to prove

any relation between consumption of tansy ragwort and Pictou disease.

To disprove the theory that Pictou disease was contagious, as decided upon by the Canadian government study, Pethick experimented with cattle in 1903. Control animals were fed hay from tansy ragwort-free Quebec, while being housed in an old stable in which death from tansy ragwort poisoning had previously occurred. Sixteen other animals were housed in a new stable while being fed hay from the contaminated area around Pictou. Controls remained normal until slaughter after 23 months of experiment. Innoculation with blood from diseased animals also failed to cause any symptoms or lesions. Contrastly, 15 of the 16 animals consuming tansy ragwort died with typical lesions. It was also noted that sheep were unaffected by grazing ragwort infested pasture except for yellowing of the flesh.

Little is known about the introduction of tansy ragwort to the Pacific Northwest. Earliest recorded mention of the plant was 1913 from Nanaimo, British Columbia (Isaacson, 1973). In 1922, tansy ragwort was discovered in a ballast dump in Portland, Oregon. However, livestock death in Oregon was not attributed to this plant until 1950 (Snyder, 1972).

Distribution of Pyrrolizidine Alkaloids

Many species of plants contain pyrrolizidine alkaloids. These plants are found within the families Boraginaceae, Compositae, Gramineae, Leguminosae, Orchidaceae, Rhizophoraceae, Santalaceae, and Sapotaceae (Bull *et al.*, 1968). Although many plants in these families do

not contain pyrrolizidine alkaloids, all Senecio (family Compositae) and Crotalaria (family Leguminosae) species as well as all plants in the Heliotropioideae and Boraginoideae subfamilies have had pyrrolizidine alkaloids isolated from them.

While mostly of the herbaceous variety, some alkaloid-containing plants are shrubs and climbers, and a few are trees. All climates and areas of the world have representatives of these plants.

Of special interest in Oregon are Senecio jacobea L. (tansy ragwort) and Senecio vulgaris L. (common groundsel) because of their prevalence in animal grazing areas. Senecio jacobea is a biennial plant with green leafy stem and yellow clustered flower head (Kingsbury, 1964). The seedling stage begins in the fall, forming a rosette the first season. The second year, the plant attains its full height of one to four feet. Seeds are carried by the wind (Gilruth, 1903).

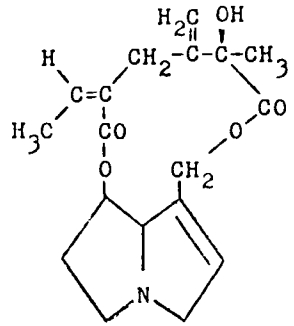
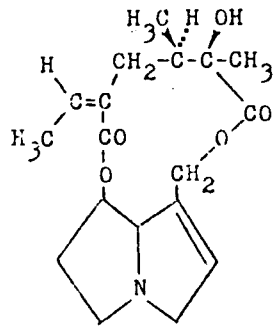
Senecio vulgaris is a low annual herb, also with green leafy stem and yellow flowers. It is common from California northward (Stuhr, 1933).

Senecio jacobea contains six pyrrolizidine alkaloids: Seneciphylline, senecionine, jacobine, jacoline, jaconine, and jacozine (Bull et al., 1968). Structures and pertinent properties of these alkaloids are listed in Table 1.

Metabolism of Pyrrolizidine Alkaloids

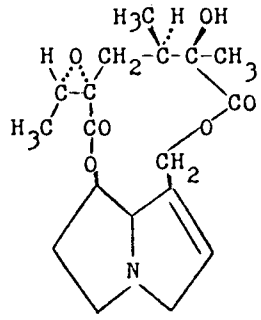
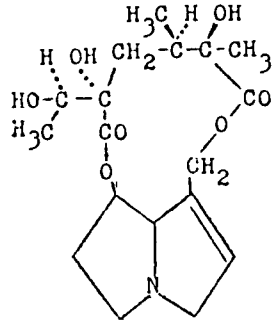
The intact alkaloids themselves are very stable. Most likely, alkaloids undergo metabolism to a more reactive form which is responsi-

TABLE 1. PYRROLIZIDINE ALKALOIDS IN SENECIO JACOBEA (TANSY RAGWORT)

Alkaloid	Structure[a]	% dry wt.[b] young plant	LD_{50} rat[a] (mg/kg)	pK in 80%[a] MCS[ad]
Seneciphylline		0.036	83	6.20
Senecionine		c	85	6.73

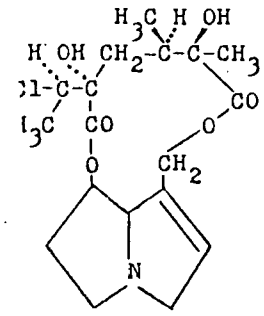
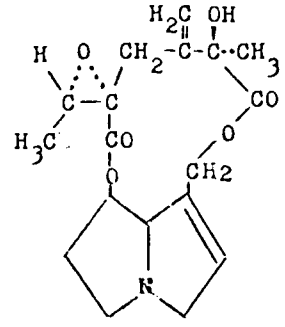
a Bull et al., 1968
b Bradbury and Culvenor, 1954
c no data available
d 80% MCS = 80% methyl cellosolve/water

TABLE 1. (continued)

Alkaloid	Structure[a]	% dry wt.[b] young plant	LD_{50} rat[a] (mg/kg)	pK in 80%[a] MCS[ad]
Jacobine		0.078	138	6.04
Jacoline		0.009	c	6.73

a Bull et al., 1968
b Bradbury and Culvenor, 1954
c no data available
d 80% MCS = 80% methyl cellusolve/water

TABLE 1. (continued)

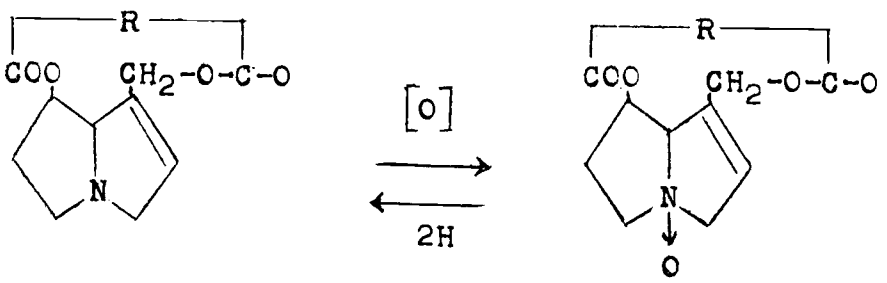
Alkaloid	Structure[a]	% dry wt.[b] young plant	LD_{50} rat[a] (mg/kg)	pK in 80%[a] MCS[ad]
Jaconine		0.018	168	c
Jacozine		0.035	c	5.96

a Bull et al., 1968
b Bradbury and Culvenor, 1954
c no data available
d 80% MCS = 80% methyl cellosolve/water

ble for the toxic effects. Mattocks (1972 b) cites several factors in support of this:

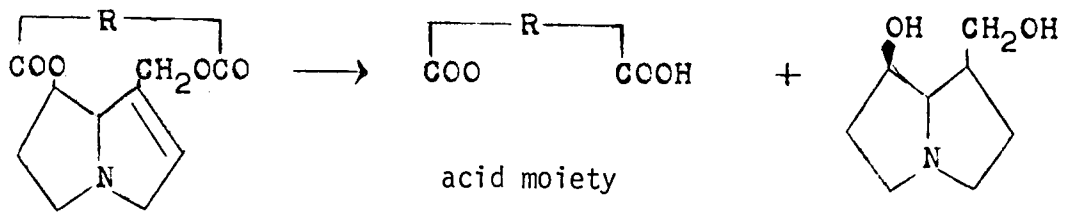
1. Regardless of the method of introducing alkaloid into the system, certain specific organs are always affected in susceptible species.
2. No reaction is evident when alkaloid is applied to the skin.
3. Some organisms are completely resistant to alkaloid poisoning and tend to accumulate alkaloid in their systems (Cinnabar moth).
4. Newborn rats are relatively resistant, possibly linked to the low activity of their liver microsomal enzymes.
5. Some species are more resistant than others.
6. Certain metabolites of the alkaloids cause damage at much lower doses than the alkaloids themselves.

The intact alkaloid may undergo one of several reactions. Alkaloid derivatives can be obtained via reversible reaction to the N-oxide form (Bull et al., 1968; Chesney and Allen, 1973; Chesney et al., 1974; Mattocks, 1972 b). This naturally occurring reaction yields a compound that is very water soluble (can be excreted in the urine), and is less toxic than the parent alkaloid (Mattocks, 1972 b). N-oxide formation, therefore, may be a mechanism by which the body rids itself of a toxic material (Chesney and Allen, 1973).



parent alkaloid N-oxide

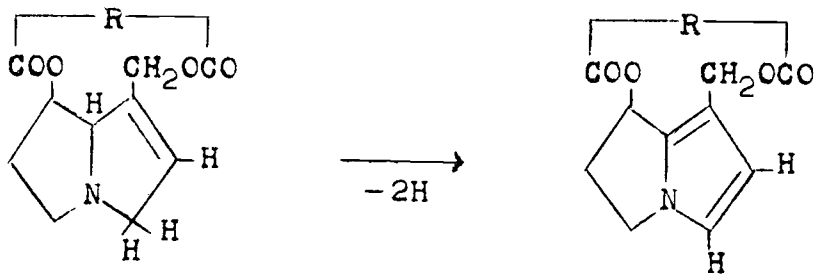
Another possible pathway is alkaline hydrolysis (Mattocks, 1972 b), in which the ester groups are removed (Bull et al., 1968). The acid and base moieties left are both non-toxic (Schoental, 1968).



parent alkaloid base moiety

This pathway will be discussed later as a possible detoxication mechanism.

The reaction now believed to be responsible for producing the proximate toxin is that of dehydrogenation (Chesney et al., 1974). The metabolite formed is termed "pyrrole" (Bull et al., 1968; Mattocks, 1968 a). The liver microsomal enzyme system plays a role here (Allen et al., 1972).



parent alkaloid pyrrole derivative

The pyrrole metabolite can serve as a bifunctional alkylating agent. The heterocyclic part of the molecule is capable of binding to nucleophilic tissue, such as liver (Mattocks, 1970). These bound pyrroles, if soluble, can be excreted in the urine or absorbed (Mattocks, 1968 a). Pyrroles may also be carried in the blood to other nucleophilic tissues before becoming bound. Pyrroles will remain bound for 48 hours or more, after which either all or part of the molecule is removed. Removal may be due to breakdown of the tissue to which it is bound.

The non-heterocyclic portion of the pyrrole molecule, or acid moiety, may influence the molecule's distribution within the body, its affinity for metabolizing enzymes, and may facilitate hydrolysis (Mattocks, 1970). With this in mind, the relative toxicities of different pyrrolizidine alkaloids may be explained by their various acid moieties.

A procedure for detecting and quantatively estimating pyrrolic derivatives formed from parent alkaloids has been established by Mattocks (1967, 1968 b). A modified reaction using Ehrlich reagent is specific for pyrroles. Liver tissue can be measured as to its bound pyrrole con-

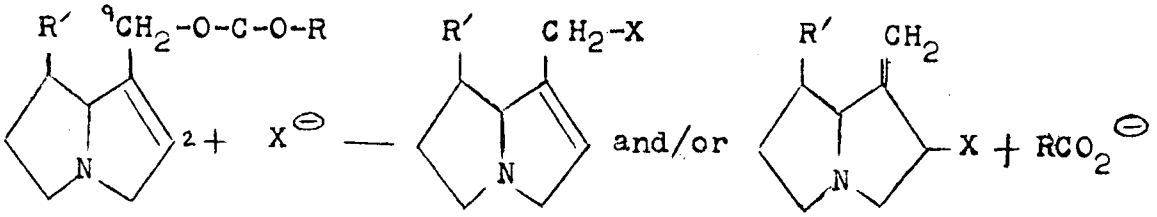
tent after injection or consumption of pyrrolizidine alkaloids.

The "two hour bound pyrrole test" is an assay available for showing the relationships between injected alkaloid and hepatotoxicity (Mattocks and White, 1970; Mattocks, 1972 a). The maximum pyrrole level in the liver is present two to five hours following injection (Mattocks, 1968 a). A linear relationship is established between dose of alkaloid and pyrrole level (Mattocks and White, 1970). The amount of pyrrolic metabolite formed is roughly proportional to the toxic effects exhibited (Mattocks, 1972 a).

Presence of pyrrolizidine alkaloids in plants can be determined by extracting the plant with methanol to isolate the alkaloids (Mattocks, 1967). The alkaloids are oxidized to their N-oxide form, and converted to the pyrrole by treatment with acetic anhydride. The pyrrolic form is then available for a colorimetric reaction with 4-dimethylamino benzaldehyde (Erlich reagent).

Metabolic Protection Against Pyrrolizidine Poisoning

It was previously believed that alkaloids acted as alkylating agents, and exerted toxic effects on cell nuclei in this way. Culvenor *et al.* (1962) proposed that certain pyrrolizidine alkaloids could undergo alkyl-oxygen fission of their ester linkage. This reaction involves the formation of an electrophilic grouping at position C2 or C9. Nucleophilic compounds (tissue constituents) will become bound.



Although this theory has since been replaced with the pyrrole theory in terms of toxic action, it may be applicable when offering an explanation for certain detoxication mechanisms. Culvenor et al. (1962) also noted that benzyl mercaptan, a very nucleophilic thiol compound, served to replace the anion lost from the parent alkaloid during alkyl-oxygen fission. They extended this idea by using the nucleophile cysteine as a protective agent, since this sulphur amino acid would preferentially combine before tissue nucleophiles.

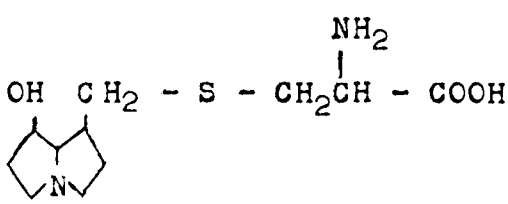
In pursuit of this theory, Hayashi and Lalich (1968) followed injections of monocrotaline into rats with an injection of either mercaptoethylamine or cysteine, and noted survival time and rate of growth. Both parameters were improved by these thiol compounds, with mercaptoethylamine being slightly more effective. A more recent study showed 1% cysteine, 1% methionine, and high dietary protein increased survival time and improved growth in rats fed 10% tansy ragwort (Cheeke and Garman, 1974). Five percent glutamate also slightly increased survival time and growth. This response may have occurred because glutamate can be transaminated to serine which is necessary for the conversion of

methionine to cysteine, cystine, and inorganic sulfate. When comparing 1% methionine to 3% ammonium sulfate and 3% ammonium sulfate plus 1% arginine, as dietary additivies for rate consuming 5% tansy ragwort, methionine fed rats gained twice that of control rats. However, methionine did not prolong survival time.

Buckmaster et al. (1976) established a partial protective effect of 1% cysteine against 10% tansy ragwort in the diet. One percent cysteine also increased survival time in rats injected with acute doses of pyrrolizidine alkaloids. White (1976) found pre-treatment with cysteine lowered the quantity of liver pyrroles present 2 hours after injection of the alkaloid retrorsine by 60%.

Glutathione has also been examined as to its protective role in pyrrolizidine alkaloid poisoning (White, 1976). Liver glutathione levels were manipulated to be either 25% or 200% that of control rats. In those rats with increased liver glutathione, the retrorsine LD_{50} was doubled. The LD_{50} was significantly lowered in rats with less glutathione. Injection of alkaloid did not cause a change in liver glutathione levels until 24 hours after dosing, at which time amounts increased relative to that of control animals.

A contrasting view is held by Robertson and Seymore (1976), who incubated dehydroretronecine with cysteine and glutathione and obtained 9-thio-cysteine dehydroretronecine as the major reaction product.



These investigators feel this compound is indicative of a route for toxic action.

In sheep, detoxication of heliotrine to 7-α-hydroxy-1-methylene-8-α-pyrrolizidine was enhanced by the addition of vitamin B_{12} to rumen contents (Dick et al., 1963). Other alkaloids incubated in vitro with B_{12} and ovine rumen fluid were also changed to non-toxic 1-methylene derivatives. Decreasing pH lessened the conversion rate.

When vitamin B_{12} was added to rumen fluid from pen-fed sheep receiving alfalfa and oaten chaff, no increase in vitro heliotrine metabolism was noted (Lanigan, 1970). Rumen fluid from sheep at pasture, however, showed increased detoxication activity with the addition of 2 mg/ml cyanocobalamin. Removal of coarse fluid constituents through centrifugation greatly decreased conversion to the non-toxic 1-methylene derivative, even with additional vitamin B_{12}, indicating the presence of another influence factor.

Lanigan (1971) also noted that in vitro heliotrine metabolism was increased with the addition of hydrogen gas. Vitamin B_{12} may induce formation of a metabolic form of hydrogen necessary for heliotrine reduction.

Shull et al. (1976 b), found that tansy ragwort incubated in sheep rumen fluid, then freeze dried and fed to rats, was more toxic than tansy soaked in cattle rumen fluid. Autoclaving either species' rumen fluid before incubation with tansy ragwort had protective effects as assessed by survival time. Effects on pH of the solutions were suggested as factors involved in this protective activity.

Organs Affected by Pyrrolizidine Damage

Upon injection, distribution of pyrrolizidine alkaloids in the body follows a consistent pattern. After one hour, regardless of injection site, most of the alkaloid will have been removed from the blood (McLean, 1970). Within three hours after injection, up to 80% of tritium labelled alkaloid can be recovered intact from the urine. Thirty percent of the label which is not complexed with alkaloid appears in the bile, and 10% in expired CO_2.

Six hours after injection of a pyrrole derivative of one of the pyrrolizidine alkaloids, label will have accumulated in significant amounts in rat's stomach, liver, skin (hair follicles), spleen, bone marrow, and kidney. Small quantities of label are found in the adrenals, bladder, brain, heart, lungs, muscle, lymph nodes, testes, pancreas, and thymus (Shumaker *et al.*, 1976). Bull *et al.* (1968) also found label in the intestines and a small amount in the brain of rats injected with lasiocarpine.

Development of lesions in specific organs varies with species, particular alkaloid used, amount and method of dose, sex, and age (Schoental, 1968). Species differences will be fully discussed in a section devoted solely to that purpose.

Liver damage is the classic pathological condition associated with pyrrolizidine alkaloids. Acute hepatic lesions developing after the LD_{50} dose include centribobular and periportal necrosis, and hemorrhage into the lesion (McLean, 1970). Hepatocyte nuclei are also affected via a drop in DNA-mediated RNA synthesis as well as decreased

cytoplasmic protein synthesis.

Repeated smaller doses of alkaloid, or alkaloid obtained through ingestion of poisonous plants over a period of time, result in chronic liver lesions. Signs of chronic damage are megalocytosis of parenchymal cells (enlargement due to failure of cells to divide), bile duct proliferation, fatty changes, fibrosis, cirrhosis, and vascular lesions. Cytoplasmic nuclear invaginations have been noted in mouse hepatocytes (Hooper, 1974). Campbell (1956) also found sinusoidal congestion and endothelial disruption in livers.

Gross appearance of pyrrolizidine alkaloid poisoned livers is granular (Mohabbat et al., 1976; Van Es et al., 1929) with a firm, thickened, sometimes yellowed capsule (Gilruth, 1902; Gilruth, 1904; Van Es et al., 1929; Butler et al., 1970).

Chronic lung lesions are observed following doses of monocrotaline pyrrole, retrorsine pyrrole (Butler et al., 1970), monocrotaline, fulvine (Barnes et al., 1964), and ingestion of Crotalaria spectabilis (Allen, 1963) and Senecio jacobea (Hooper, 1974). Other pyrrolizidine alkaloids and their derivatives are capable of producing chronic lung lesions, but at dosage levels somewhat higher than those causing liver damage (Culvenor et al., 1976).

Typical lung lesions are pulmonary edema, congestion, hemorrhage, arteritis (McLean, 1970; Allen, 1963; Butler et al., 1970), epithelial hyperplasia with papillary outgrowths, patchy emphysema (Bull et al., 1968), cytoplasmic invaginations, and enlarged bronchial epithelium cells (Hooper, 1974; Harding et al., 1964).

Occasionally kidney tissue is affected by pyrrolizidine alkaloid poisoning. Epithelial cell enlargement in both the proximal tubule and loop of Henle was noted by Hooper (1974) in mice. Hypertrophy of convoluted tubule epithelial cells was evident in rats (Bull et al., 1968). Glomerular atrophy, metaplastic changes in Bowman's capsule, and luminal blockage of kidney tubules have also been noted (Van der Watt et al., 1972).

Pyrrolizidine alkaloid metabolites also cause gastrointestinal damage (Hooper, 1975). Lesions were most often seen in the glandular portion of the stomach, duodenum, and jejunum. Cells were enlarged, mitoses were abnormal, and ulceration was evident.

Species Differences In Response To Pyrrolizidine Alkaloids

(a) Cattle

Extreme variability is evident between species in their susceptibility to pyrrolizidine alkaloid poisoning. As previously indicated, cattle succumb rapidly to the toxic action of pyrrolizidine alkaloids. Survival time is related to the amount of plant consumed. In a study by Thorpe and Ford (1968), young Ayrshire X Shorthorn male calves were fed pellets containing 20% tansy ragwort. The trend was for those calves consuming a greater amount of tansy to have a shorter survival time. Hepatic lesions, including megalocytosis, veno-occlusive lesions, and fibrosis became more severe as survival time increased.

After consumption of alfalfa hay contaminated with Senecio vulgaris, twelve dairy heifers suffered ataxia, prolapsed rectum, and con-

gested mucus membranes (Fowler, 1968). The very firm livers showed megalocytosis and excess fibrosis. Deaths of calves from the same herd, which had not consumed the contaminated hay, but whose mothers had while pregnant, indicated passage of the alkaloid to the fetus. Another clinical study reported symptoms of a one and one half year old heifer exposed to tansy ragwort (Tilt, 1969). Weakness, emaciation, dry hair coat, rapid breathing, distended bladder and periodic straining were visible signs. Pathologically, heart and lung hemorrhages, a thickened gall bladder wall, and liver showing fibrosis, bile duct proliferation and megalocytosis were seen.

Sucking calf studies were conducted by Johnson (1976) to examine the possibility of alkaloid transfer through the milk. Cows were fed tansy ragwort beginning within 30 days post partum. Calves were allowed to nurse until their mother's death. The cows' liver damage was that typical of pyrrolizidine alkaloid poisoning. Calves, contrastly, appeared grossly normal. The liver of only one calf had any sign of microscopic change. However, all calf blood data showed increased lactate dehydrogenase (LDH) and glutamate-OAA transaminase (GOT) values, indicating possible pyrrolizidine alkaloid poisoning. Other measureable blood parameters indicative of pyrrolizidine alkaloid poisoning in calves are sorbital dehydrogenase (SD), ornithine carbamyl transferase (OCT), and hyperbilirubinemia (Ford *et al.*, 1968).

A similar experiment was conducted by Dickinson *et al.* (1976), in which lactating cows consumed 10 g of dried tansy ragwort per kg body weight per day. Alkaloids were found in the milk, but the calves were

not affected.

Thorpe and Ford (1968) found typical pathologic signs upon post mortem examination of calves that had consumed tansy ragwort. These included large amounts of yellow edema, enlarged liver with a thickened opaque capsule, and pale hepatic parenchyma. Parenchymal cell megalocytosis, steatosis, centrilobular necrosis, veno-occlusive lesions leading to pooling of blood, fibrosis, and duct proliferation were also seen. In addition to these signs, dropsy, bloody feces, and hemorrhages in the liver, stomach and intestine are common (Cushny, 1911).

(b) Horses

Death of horses has been associated with pyrrolizidine alkaloids since the 1890's. More recently, clinical symptoms have been accurately noted (Rose et al., 1957). A horse will exhibit a desire to walk a straight line in spite of obstacles. The animal will stand with its head against the object blocking its path with an inability to turn in either direction. As a result of this determination to walk a fixed course, drowning is common. Upon autopsy, the liver appears smaller than normal with cirrhotic changes. Blood ammonia levels rise just before the onset of "Walk-About" disease, indicating toxication of the nervous system. Oddly, only domestic equines seem to be affected. No cases with these symptoms in wild horses or donkeys have been reported.

Van Es et al. (1929) reported on the Walking Disease of horses in Northwestern Nebraska. Evidence of other cases came from neighboring Wyoming and Colorado. Cattle dying from a similar disease were also the object of study. Symptoms described for horses included those

noted by Rose as well as yawning and a decreased appetite. Mucus membranes and subcutaneous tissue appeared yellowed. The stomachs of afflicted horses were dilated. Livers were smaller than normal, mottled (nutmeg effect), very firm and exhibited necrosis and megalocytosis upon microscopic examination.

(c) Sheep

As mentioned previously, sheep can graze pasture heavily infested with Senecio jacobea with apparent resistance the first season (Gilruth, 1903 and 1904). In a feeding experiment, Bull et al. (1956) found that sheep grazing Heliotropium europaeum remained, for the most part, healthy the first year, but suffered high death losses during the second grazing season. Jaundice, hemoglobinuria, and abnormally high liver copper levels were present in many of the animals. Smaller than normal livers contained inclusion globules. A second similar experiment by Bull et al. (1956) confirmed these results.

Sheep injected intraperitoneally with lasiocarpine experienced severe hepatic necrosis. Lesions were also evident in the gall bladder, central nervous system, kidney, abomasum and small intestine (Hooper, 1975).

Sheep drenched with a one pound dose of Crotalaria mucronata died of acute pyrrolizidine alkaloid poisoning (Laws, 1968). Large quantities of yellow fluid were found in the thoracic, pericardial and abdominal cavities. Kidneys were congested and the duodenum showed lesions; however, no other microscopic lesions were visible in any of the commonly affected organs.

The major breakdown products present in ovine rumen fluid after dosing with heliotrine were found to be heliotric acid and 7-∝-Hydroxyl-1-methylene-8-∝ pyrrolizidine (Russell and Smith, 1968). Metabolites of heliotrine present in the urine and bile of sheep are heliotridine, heliotridine trachelanthate, heliotrine N-oxide, and unchanged heliotrine (Jago et al., 1969). These metabolites are not as toxic as heliotrine itself.

Lanigan (1970) determined that the microorganisms in ovine rumens necessary to metabolize heliotrine to a non-toxic form may not be present in all sheep due to a lack of specific substrate. The bacteria will proliferate, however, if the animal is slowly introduced to a substrate such as Heliotropium europaeum.

Recently, the specific organism responsible for reductive breakdown of Heliotrine in sheep has been isolated (Lanigan, 1976). The new species has been named Peptococcus heliotrinreducans. Besides heliotrine, the organism is also responsible for cleavage of europine, heleurine, supinine. Alkaloids that are cyclic diesters, as are all six of those found in tansy ragwort, are broken down to a slower rate than the afore-mentioned monoesters.

Peptococcus heliotrinreducans is in competition with rumen methanogenic bacteria for molecular hydrogen (Lanigan, 1976). Lanigan (unpublished results) fed sheep iodoform, a methanogenesis inhibitor, in a ration containing 50% Heliotropium europaeum. The iodoform, in theory, should eliminate Peptococcus heliotrinreducans' competitor enough to allow increased metabolism of the alkaloids. In one trial,

however, the iodoform level was too high, causing hepatoxic effects due to the iodoform itself. In another trial, 31 mg of iodoform given twice daily delayed signs of liver damage, but was not fully protective.

(d) Swine

Swine consuming plants containing pyrrolizidine alkaloids suffer damage in kidney and lung tissue to a greater extent than the liver. Harding et al. (1964) conducted two experiments in which young pigs were fed tansy ragwort at 5% and 10% levels in their normal diet until death or slaughter. Several pigs were not affected as confirmed by normal organs found in examination after slaughter. Other pigs died after an average of 70 days. On these animals, livers were relatively normal. Lungs, however, showed pulmonary edema, congestion, hemorrhage, and alveolar epithelialization; and kidneys were noted to have enlarged epithelial cells in the proximal convoluted tubule.

Bull et al. (1968) obtained similar kidney lesions when feeding tansy ragwort or Heliotropium europaeum to swine. No severe lung lesions were noted, but pigs consuming Heliotropium europaeum suffered advanced liver damage.

A clinical report presented a case in which 76 of 150 swine died from eating corn contaminated with Crotalaria spectabilis seeds (Peckham et al., 1974). Loss of back hair was the first symptom noticed; anemia and weight loss followed. Most deaths occurred eight to twelve weeks after consumption of the contaminated grain. Lung and kidney lesions were prevalent. Gastric ulcers, and hepatic fibrosis and atrophy were also noted. It was observed that weaner pigs were much

more susceptible than pigs near slaughter weights; with mortality rates of 80 to 8%, respectively. Swine fed whole Crotalaria spectabilis seeds died within 57 days, depending on dose (Emmel et al., 1935). Chronic lesions were suffered in heart, kidney, liver, lung, and stomach tissue after symptoms previously described were exhibited. Hooper (1977) has expressed relative chronic toxicities of ingested tansy ragwort to several animal species (Table 2). Table 3 shows relative chronic toxicities of ingested monocrotaline. In both cases, pigs were very sensitive to pyrrolizidine alkaloid poisoning.

(e) Avian Species

Pyrrolizidine alkaloids have various effects on fowl, depending on species. Turkeys are very susceptible to poisoning by seeds from Crotalaria spectabilis, a plant containing the pyrrolizidine alkaloid monocrotaline. Lethal doses of alkaloid for poults can be as small as 0.125% to 1.0% of the diet (Allen, 1963). Typical histological changes occur in the liver; necrosis, bile duct proliferation, destruction of lobular structure, thickened capsule, and fibrosis (Allen, 1963; Allen et al., 1963).

Chickens fed Crotalaria seeds also succumbed. In addition to liver congestion, the spleen was atrophied, pale, and congested, the gizzard discolored by bile, intestine inflamed and atrophied, the lungs appeared congested, edematous, and hemorrhaged (Emmel, 1937). Of 43 chickens fed 4% tansy ragwort in a standard chick mash, half died in 14 weeks. The liver capsule was thickened; and necrosis, congestion and hemorrhage were evident. Other chickens were injected with 20-80

TABLE 2. RELATIVE CHRONIC TOXICITY OF SENECIO JACOBEA TO FIVE SPECIES[a]

Species	Kg tansy ragwort/kg body wt.
Mice	1.5
Rats	0.5
Cattle	0.14
Chickens	0.05
Swine	0.005

a Hooper (1977)

TABLE 3. RELATIVE CHRONIC TOXICITY OF MONOCROTALINE TO EIGHT SPECIES[a]

Species	Kg tansy ragwort/kg body wt.
Sheep, Goats	200*
Mice	150
Rats	50
Horses, Cattle	14
Chickens	5
Swine	1

* Relative lethal dose, with swine given an arbitrary value of 1.

a Hooper (1977)

mg/kg alkaloid extracted from tansy ragwort. The LD_{50} was determined to be about 40 mg/kg body weight (Campbell, 1956).

Quail are different yet in their susceptibility to pyrrolizidine alkaloid toxicity. Birds force fed 40 Crotalaria spectabilis seeds became ill (Thomas, 1934). Japanese quail (Coturnix coturnix japonica) allowed to eat 10% tansy ragwort in a standard layer mash consumed the mixture for 365 days with no ill effects (Buckmaster et al., 1977).

(f) Rabbits

Rabbits have been fed a variety of pyrrolizidine alkaloids containing plants. Van Es et al. (1929) reported experimental feeding of Senecio riddellii to four rabbits. Senecio riddellii contains the alkaloid riddelline. After 42 days, the animals appeared normal. Histologically, two rabbits had advanced lesions and fibrosis. The other animals were affected only slightly. Van Es et al. (1929) also fed Senecio integerrimus, containing integerrimine and senecionine, to four rabbits for 55 days. On day 81, the animals were sacrificed. Although grossly normal, microscopically three of the livers showed minor irritations.

A rabbit fed Crotalaria crispata died after 136 days (Bull et al., 1968). This plant contains monocrotaline, fulvine, and crispatine. The liver showed signs of megalocytosis. Kidneys were affected; proximal convoluted tubule epithelial cells were enlarged with large nuclei. Lungs showed patchy emphysema and enlarged alveolar cells.

(g) Deer

Only one account of experimental feeding with deer was found. Black-tailed deer were grouped as to amounts of Senecio jacobea available in the diet for 42 days (Dean and Winward, 1974). Palatability problems were encountered; resulting in having only the group whose regular diet was very restricted, eat 24% of their body weight in tansy ragwort. No liver lesions were found in any of the animals. Taste preferences seem to be a natural form of protection for deer.

(h) Humans

Humans are also subject to pyrrolizidine alkaloid poisoning. Clinical reports on Senecio contamination of bread and tea have been issued from Africa, India, and the West Indies (Selzer and Parker, 1951; McLean, 1970; Mohabbat et al., 1976; Tandon et al., 1976). Veno-occlusive disease is evident in under-developed countries in years of drought when pyrrolizidine alkaloid containing plants survive better than crops. This disease, also called Chiari's syndrome, causes the liver to take on a granular appearance; hemorrhage, necrosis, fibrosis, and centrilobular congestion are also exhibited. Symptoms include abdominal pain, distended abdomen, ascites, and death. In the United States, ingestin of herbal teas prepared from Senecio longilobus has been fatal to children. The plant contains the alkaloids riddelliine, retrorsine, seneciphylline and senecionine. If the first dose of tea did not cause death, successive intake caused ascites and liver enlargement (Huxtable et al., 1977).

(i) Non-Human Primates

Non-human primates have been tested as to their relative susceptibility to pyrrolizidine alkaloid poisoning and lesions produced. Van der Watt et al. (1972) dosed male Vervet monkeys intragastrically with the alkaloid retrorsine. After 30 treatments, one per week, hepatic veno-occlusive disease and kidney damage were noted.

Another study involved two age groups of Macaca arctoide monkeys (Allen and Chesney, 1972). One group of 30 day old infants survived an average of 241 days. Symptoms of poisoning included increases in hemoglobin levels, hematocrits and blood pressure, bilateral myocardial hypertrophy, fibrous and edematous myocardium, right heart dilation, and firm lungs with thickened pulmonary arteries, some with obstructed lumens. The other group, consisting of adolescents 15 months of age, survived 217 days on the average. Symptoms typical of this group were also different; ascites, distended abdominal veins, and increased portal venous pressure. Damage including veno-occlusive disease and fibrosis of small, firm, granular livers was evident. It was suggested that differences between the two groups were due to liver microsomal enzyme activity levels. Infant enzyme activity had not yet reached the level of the adolescent. Adolescent monkeys produced more metabolic pyrroles, thought to cause direct liver damage.

(j) Rats

Rats are frequently used as pilot animals in pyrrolizidine alkaloid studies. They are susceptible to both injected and ingested alkaloid. In feeding experiments with 8% Senecio jacobaea or 8% Helio-

tropium europaeum, rats gained weight poorly (Bull et al., 1968). Those consuming Senecio jacobaea lived about two months. Livers from these rats had developed megalocytosis, vascular congestion and hemorrhage. Survival time was considerably longer for the Heliotropium europaeum group. Rats were sacrificed after 83 days, with livers revealing more extensive megalocytosis. Kidneys were normal in both groups.

In an experiment in which rats consumed tansy ragwort as 10% of their diet, livers appeared very dark in rats sacrificed on day 15 and 31, but were light colored by day 45 (Buckmaster et al., 1976). Spleen weights were above normal at day 45. A possible explanation for such changes may be the storage of iron from red blood cell destruction at first in the liver, which is later transferred to the spleen.

Rats consuming fixed amounts of tansy ragwort were examined in a study by Cheeke and Garman (1974). Animals whose tansy ragwort intakes were 15 or 20 grams had pronounced gross liver and lung damage, abdominal edema, and jaundice. As tansy intake increased, liver weight as a percent of body weight decreased. Serum albumin levels increased, serum albumin/globulin ratios decreased, and serum bilirubin levels increased with increasing tansy ragwort intake.

After consuming a diet containing 0.02%-0.08% Crotalaria spectabilis seed for 8 months, rats developed lung lesions including hydrothorax and edema (Allen and Carstens, 1970). Kidneys and livers were mildly damaged.

Buckmaster et al. (1976) compared the effects of feeding rats a

diet containing 5% tansy ragwort to one containing 5% common groundsel. Total alkaloid intake was less for those animals consuming groundsel; weight gain and survival time were also less than that of the tansy ragwort fed group. Based on these results, groundsel alkaloids were about 1.5 times more toxic than tansy ragwort alkaloids.

Median lethan doses (LD_{50}) for many pyrrolizidine alkaloids have been determined with rats. Male rats injected intraperitoneally with monocrotaline had an LD_{50} of 109 mg/kg; retrorsine: 34 mg/kg; senecionine: 50 mg/kg (Mattocks, 1972 a); and seneciphylline: 80 mg/kg (Anon, 1949). Tissue damage associated with acute deaths includes hemorrhagic necrosis and congestion of the liver, ascites, and pulmonary edema.

Shull et al. (1976 a) reported the effects of a single non-lethal injection of alkaloids extracted from tansy ragwort. Rats' pyrrole production was reduced within one hour after injection, possibly due to direct binding of pyrroles to the enzymes involved in their own production. Levels of amino-pyrine N-demethylase, a liver microsomal enzyme involved in hydroxylation of foreign compounds, and cytochrome P-450, also involved in hydroxylation reactions, were reduced within 24 hours. Spleen weights showed enlargement until day 6, but declined thereafter, reaching normal values by day 57 following injection.

Buckmaster et al. (1976) compared the toxicities of alkaloids isolated from common groundsel and tansy ragwort. The LD_{50} was lower for groundsel alkaloid. This effect was attributed to the greater amount of seneciphylline, which is very toxic, being present in groundsel.

(k) Mice

Mice are susceptible to pyrrolizidine alkaloid poisoning, but to a lesser extent than rats. Young male mice were fed 10% tansy ragwort in their diet for nine weeks, then 20% tansy until death (Hooper, 1974). Lesions were not apparent through day 129. After that, edema was obvious. Livers of autopsied mice showed megalocytosis and cytoplasmic invaginations of hepatocytes. Fibrosis and veno-occlusive disease were not evident. In addition to liver lesions, lungs and kidneys of mice were affected. Megalocytosis and cytoplasmic invaginations of lung epithelial cells and alveolar cells were common. In the kidney, epithelial cells in the proximal tubule and loop of Henle were slightly enlarged.

Mice injected intraperitoneally with the alkaloid lasiocarpine exhibited histological lesions of the gastrointestinal tract (Hooper, 1975). Stomach and duodenal cysts with cytoplasmic invaginations developed, as well as enlarged duodenal cells.

(l) Guinea Pigs

Schoental (1968) and McLean (1970) stated that liver tumors due to pyrrolizidine alkaloid poisoning had not been induced in guinea pigs. However, guinea pigs injected intravenously with a 2% solution of seneciphylline in doses ranging from 50-80 mg/kg, died after convulsions (Chen et al., 1940). Those guinea pigs surviving 30-35 days were observed as chronic alkaloid poisoning cases. Two of 15 animals which died showed signs of slight centrilobular necrosis of the liver. Kidneys were also affected by cloudy swelling, interstitial infiltration of leucocytes and a crescent-like structure of the glomercular capsule.

Reticulo-endothelial hyperplasia of the spleen was noted in several guinea pigs.

In a previous experiment, Chen et al. (1935) intravenously dosed guinea pigs with 160 mg retrorsine/kg on four successive occasions. Histological examination of animals sacrificed after ten days revealed no pathological changes. White et al. (1973) found guinea pigs to be relatively resistant to a dose of 800 mg retrorsine/kg. This dose was sufficient to cause centrilobular hemorrhagic necrosis, but failed to kill the animals. Injection of 380 mg jacobine/kg caused fibrosis and bile duct proliferation of the liver, but not death (Hopkirk and Cunningham, 1936).

Guinea pigs were injected with four times the monocrotaline LD_{50} established for rats (Chesney and Allen, 1973). Those guinea pigs were compared to rats in their susceptibility to toxic effects, pyrrole production, and N-oxide production. For six weeks following the injection, guinea pigs remained normal and no changes were discovered at necropsy. Pyrrole production in guinea pigs was 30 times slower than in injected rats, while N-oxide production was greater. This data suggested that the microsomal enzyme system responsible for forming pyrroles from parent alkaloids is not present in guinea pigs, but the N-oxide producing system is very active. Both systems are active in the rat. Since the N-oxide form is non-toxic, this may be a detoxifying mechanism unique to the guinea pig.

Ground Crotalaria spectabilis seeds containing monocrotaline incorporated at the 1% level into guinea pig diets had no clinical effect

after seven months of feeding (Carlton, 1967). However, when the level of seeds was raised to 5% of the diet, guinea pigs developed ascites and pathologic changes in the liver and lungs. Megalocytosis, congestion and dilation of lymphatics were noted in livers at autopsy. Lungs had focal areas of pneumonia and congestion.

(m) Hamsters

Hamsters were found to have the highest rate of pyrrole production of all animals tested by Shull et al. (1976 b). Pyrrole production rates were several times greater in male hamsters than in male rats. Both monocrotaline and tansy ragwort mixed alkaloid were used.

Rose et al. (1959) reported hamsters to be more susceptible to injected alkaloid than rats and mice. The LD_{50} for lasiocarpine was 67.5 $\pm$ 5.6 mg/kg in golden hamsters, while for mice and rats the LD_{50} value was 85.1 $\pm$ 2.9 and 88.1 $\pm$ 13.2, respectively.

III. EXPERIMENTAL

Prevention of livestock mortality from tansy ragwort poisoning is of utmost importance in the Pacific Northwest. A solution to this multi-million dollar problem must be sought through research with laboratory animal models. These experiments are intended to bring a solution closer by examining a variety of laboratory animal species for their resistance and susceptibility to pyrrolizidine alkaloidosis. With this information, the mechanism of toxicity may be established. An additional aspect that was investigated was the effect of dietary additives on the susceptibility of rats to tansy ragwort toxicity.

All tansy ragwort used in these experiments was collected locally while in full bloom, air dried, and finely ground for feeding and extraction purposes.

Materials and Methods

(a) Species Differences in Response to Dietary Tansy Ragwort

Seven species were studied as to their reactions to ingested and/or injected pyrrolizidine alkaloid from tansy ragwort. Rabbits, rats, mice, gerbils, hamsters, guinea pigs, and Japanese quail were used. Although all are monogastric, several of these species have pronounced bacterial fermentation in their stomach and/or cecum. Thus, laboratory animal models might be identified that closely simulate the metabolic pathway of tansy ragwort toxicity in both cattle and horses.

Rabbit Experiment

Investigation of the effect of chronic tansy ragwort consumption

in rabbits was prompted by knowledge of their high rate of *in vitro* pyrrole production, suggesting high susceptibility (Shull *et al.*, 1976 b). If this proved to be true, rabbits could be used as a laboratory animal model for a species susceptible to tansy ragwort poisoning.

Twelve male New Zealand White rabbits of about 2 kg weight were divided into a control and a control plus tansy ragwort group. For the first 90 days, the tansy ragwort level was 5%; for the remainder of the trial it was 10%. The control diet for the first 90 days was 54.5% ground corn, 40% dehydrated alfalfa meal, 5% lard, and 0.5% mineral mix. After 90 days, the control diet consisted of 44.5% alfalfa, 40% barley, 12.5% soybean meal, 2.5% molasses, and 0.5% salt. Tansy ragwort was substituted for alfalfa in the tansy diet. Blood (5 ml/animal) was collected by heart puncture once before the experimental feeding had begun, and 7 times periodically thereafter. Total serum protein (Lowry *et al.*, 1951) and serum albumin (Sigma Method No. 630)[a] were determined on the samples.

After 263 days on the diets, the animals were killed and examined histologically.

In order to determine if rabbits were susceptible to injected pyrrolizidine alkaloid, two New Zealand White rabbits weighing 2000-2500 grams were injected intraperitoneally with a solution containing 30 mg Senecio alkaloid/ml. Doses given were equivalent to 120 mg alkaloid/kg body weight. Animals were checked for vital signs periodically after injection.

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[a]Sigma Chemical Company, St. Louis, Missouri.

Mouse Experiment

The effect of tansy ragwort consumption on the laboratory mouse was studied. Forty female Swiss-Webster mice, of 14-17 g body weight, were randomly divided into four groups. Animals were group caged according to diet. The diets (Table 4) were formulated to 18% protein, and were fed ad libitum. Treatments were: 5% tansy ragwort, 10% tansy ragwort, 5% dehydrated alfalfa, and 10% dehydrated alfalfa. Body weights were recorded weekly. Feeding continued until all tansy ragwort-fed mice had died.

TABLE 4. PERCENTAGE COMPOSITION OF DIETS USED IN THE MOUSE EXPERIMENT

Ingredient	1	2	3	4
Ground corn	62.5	58.5	60.5	54.0
Soybean meal, 45.5% protein	18.0	17.0	20.0	21.5
Alfalfa (dehy)	5.0	10.0	----	----
Tansy ragwort	----	----	5.0	10.0
Fish meal	5.0	5.0	5.0	5.0
Molasses	3.0	3.0	3.0	3.0
Corn oil	3.0	3.0	3.0	3.0
Mineral mix[a]	3.0	3.0	3.0	3.0
Vitamin mix[b]	0.5	0.5	0.5	0.5

a Jones and Foster, 1942

b Cheeke et al., 1977

Quail Experiment

The effect of feeding tansy ragwort to quail on a chronic basis was studied. Twenty Japanese quail chicks (day old) were assigned to each of a control and a control plus 10% tansy ragwort diet. The control diet consisted of a quail starter diet (Table 5) for 15 days, a grower diet (Al-Soudi et al., 1974) for 14 days, and a layer diet (Reading et al., 1976) for the rest of the experiment. Feed consumption was measured for 252 days. For the duration of the experiment, feed was offered free choice but consumption not measured. At 252 days, half of the birds were killed for histological examination. The rest were killed at 365 days, and examined histologically.

TABLE 5. QUAIL STARTER DIET

Ingredient	g/kg
Soybean oil	40
Soybean meal, 44% protein	590
Glucose monohydrate	295
Mineral mixture[a]	61
Vitamin mixture[b]	7
DL methionine	5
Glycine	2

a Fox and Briggs, 1960, with 2 mg Mo and 0.1 mg Se added per kg of diet.

b B complex, vitamin K mixture (Gordon and Sizer, 1955) supplemented with 44 IU vitamin E, 9,990 IU vitamin A, 1,995 ICU vitamin D_3, and 125 mg BHT per kg diet.

Non-Ruminant Herbivore Experiment

Four species were examined as to their relative susceptibilities to tansy ragwort poisoning. Dried ground tansy ragwort was incorporated into the natural ingredient diet used previously (Table 7). Table 6 describes the species, number of animals, initial body weight, and diet. All animals were of the male sex. Each was individually housed and fed ad libitum until all tansy ragwort fed animals had died. Feed consumption and body weights were measured. After a short trial period, all guinea pigs and hamsters were changed to a diet containing 10% tansy ragwort due to their refusal of the diet containing 30%.

TABLE 6. NON-RUMINANT HERBIVORE SPECIES FED TANSY RAGWORT DIETS

Species	No. of Animals	Diet	Initial Body Weight (g)
Rat	10	30% TR	63.8 ± 2.9
G. Pig	4	10% TR	196.5 ± 19.2
Gerbil	5	30% TR	27.0 ± 2.3
Hamster	10	Control	55.8 ± 4.7
Hamster	10	10% TR	51.2 ± 5.2

Hamsters were selected because of their high rate of *in vitro* pyrrole production (Shull *et al.*, 1976 b); guinea pigs because of their reported resistance to pyrrolizidine alkaloids (Chesney and Allen, 1973) and gerbils because of their herbivorous nature and lack of any data regard-

TABLE 7. TEN PERCENT TANSY RAGWORT DIET

Ingredient	% Diet
Ground corn	50.5
Soybean meal, 45.5% protein	30.0
Tansy	10.0
Molasses	3.0
Lard	3.0
Mineral mix[a]	3.0
Vitamin mix[b]	0.5

a Jones and Foster (1942)
b Cheeke et al. (1977)

ing them.

The response of each of these species to injected alkaloid was also investigated. Although toxicity studies have been conducted previously with rats (Buckmaster et al., 1976), this species was included as a measure of assessing the potency of the injection solutions and for comparative purposes. Alkaloid was prepared for injection as described by Shull et al. (1976 a).

A preparation containing all of the alkaloids in tansy ragwort was compared with isolated jacobine (one of the six in tansy ragwort), and monocrotaline.

Table 8 lists species, alkaloid and dose. Injections were given

TABLE 8. ALKALOID INJECTION IN NON-RUMINANT HERBIVORES (Dosage in mg Alkaloid/Kg Body Weight Are in Body of Table)

Species	Monocrotaline	Jacobine	Tansy Ragwort Composite
Rat	150 250	150 200	150
Hamster	150	125	125
Guinea Pig	500	50 100 150	50 100 150
Gerbil	100 150 250	100 150 200	100 150 200

intraperitoneally. Animals were observed for one week following administration of the alkaloid.

(b) Dietary Modification of Tansy Ragwort Toxicity

In previous work (Shull et al., 1976 b), it was noted that soaking tansy ragwort in autoclaved rumen fluid reduced its toxicity. It was suggested that the reduction in potency may have been related to alkaloid instability at alkaline pH. The effect of pH was further examined in the study. Tansy ragwort was soaked at various pH's, and then fed to rats. The effect of dietary additives which might alter the gastrointestinal tract pH was also given examined.

First Soaking Experiment

Bull et al. (1968) indicated that the degree of alkaloid ioniza-

tion will influence their rate of absorption from the gastrointestinal tract. Alkaloids will be 99% ionized at a pH two units below the pKa, and will therefore be very water soluble. Absorption will be decreased at a pH that increases a substance's dissociation (Martin and Albert, 1950).

Soaking tansy ragwort in autoclaved cattle rumen fluid proved to be very effective in reducing its toxicity to rats (Shull et al., 1976 b). Autoclaving may have increased the pH of the cattle rumen fluid by killing acid producing microorganisms.

Further examination of the pH effect was made in this experiment by soaking tansy ragwort in various solutions of different pH before incorporating the tansy ragwort into the rat diets.

Diets were prepared as follows:

A. Tansy ragwort in distilled water: 450 g tansy ragwort were soaked in 3750 ml distilled water for 48 hours.

B. Tansy ragwort in acidified distilled water: 450 g tansy ragwort were soaked in 3750 ml distilled water which had been adjusted to pH 4.0 with concentrated HCl. The pH was readjusted to 4.0 after addition of tansy ragwort. Soaking time was 48 hours.

C. Tansy ragwort in basic distilled water: 450 g tansy ragwort were soaked in 3750 ml distilled water which had been adjusted to pH 8.0 with 10% sodium carbonate. The pH was readjusted to pH 8.0 with concentrated NaOH after addition of the tansy ragwort. Soaking time was 48 hours.

D. Tansy ragwort in active cattle rumen fluid: Rumen fluid obtained from a fistulated steer was strained through cheese cloth twice, and allowed to sit for one hour in a separatory funnel. The middle layer was saved. Carbon dioxide was bubbled through 900 ml of McDougall (1948) Nutrient Buffer Solution, to which 450 g tansy ragwort, 1200 ml distilled water, and 1650 ml of active cattle rumen fluid were added. Carbon dioxide was bubbled through the mixture for three minutes. The solution was placed in an incubator at 39°C for 48 hours.

E. Tansy ragwort in autoclaved cattle rumen fluid: Cattle rumen fluid was obtained and prepared as in D. After separation, the middle layer was autoclaved for 25 minutes; 450 g tansy ragwort, 900 ml McDougall's Nutrient Buffer Solution, 1200 ml distilled water, and 1650 ml autoclaved cattle rumen fluid were mixed and treated as in D. Incubation time was 48 hours at 39°C.

F. Tansy ragwort in McDougall's Nutrient Buffer solution: 450 g tansy ragwort was added to 2850 ml distilled water and 900 ml McDougall's Nutrient Buffer Solution through which carbon dioxide had been bubbled. Soaking time was 48 hours.

All flasks were swirled four times each day during the soaking period. After 48 hours, the tansy ragwort mixtures were transferred to aluminum pans, frozen, and freeze-dried. Dry tansy ragwort was incorporated at the 5% level into a basal diet containing 38% sucrose, 23% corn starch, 20% casein, 5% lard, 4% Jones and Foster mineral mix (Jones and Foster,

1942), 2% torula yeast, 2% alphacel, and 1% vitamin mix (Cheeke et al., 1977).

Eighty male Long-Evans rats, of 70 g initial body weight, were randomly alloted (10 rats per treatment) to each of the above diets, a negative control diet containing 5% untreated tansy ragwort, and a positive control diet containing sucrose in place of tansy ragwort. Animals were individually caged, and feed was offered ad libitum. Food consumption and animal body weights were measured. Rats on the control diet were taken off the experiment after 50 days. Feeding of animals remaining on diets C and E was terminated after 95 days. Survival time of the other animals was recorded.

Second Soaking Experiment

Several dietary additives were examined as to their effect on the survival time of rats fed 10% tansy ragwort. Sixty male Long-Evans rats of 60 g weight were divided among twelve experimental diets. Animals were caged individually and fed ad libitum. Feed consumption and body weights were measured, until all tansy-fed rats had died. The basal diet consisted of 50.5% ground corn, 30% soybean meal, 10% tansy ragwort, 3% molasses, 3% lard, 3% mineral mix, and 0.5% vitamin mix.

The rationale for each treatment is as follows:

Tansy ragwort was treated with 4N NaOH at pH 8.0 because of evidence of reduction in toxicity with this treatment in Experiment 1. Soaking tansy ragwort at pH 8.0 and then acidifying it to pH 4.0 was included as a treatment to establish whether the apparent detoxifying change occurring at pH 8.0 was permanent, or if toxic properties would

resume at the lower pH. Tansy ragwort soaked at pH 4.0 was included as a negative control for the two aforementioned treatments.

Ion exchange resins were incorporated into the diets with a dual purpose. Strongly basic anion exchange resin was used in an effort to raise the pH of the gut to permit tansy ragwort to soak at a high pH inside the animal. Anion exchange resin was found to be successful in decreasing free gastric and duodenal acid in ulcer and non-ulcer patients with a concurrent rise in pH values (Wirts and Rehfuss, 1950; Greenblatt et al., 1951). A two gram dose increased the gastric pH in an ulcer patient from pH 1 to pH 5-6 (Segal et al., 1950). Rats, dogs, and humans can consume anion exchange resin without suffering toxic effects (Martin and Wilkinson, 1946).

A strongly acidic cation exchange resin was also used. Mattocks (1961) extracted pyrrolizidine alkaloids from plants with cation exchange resin. Possibly the unchanged alkaloids in tansy ragwort could be removed by the resin to lessen their availability for intestinal absorption.

Another method for regulating the pH of the gut was through the use of Tris (Hydroxymethyl) Amino methane. When combined with HCL at 37°C, as it would be in the stomach, pH values will be maintained in a range from 6.70 → 8.79 (Durst and Staples, 1972).

Mineral oil was also tested as a dietary additive. Known for its absorptive abilities (Ershoff and Hernandez, 1958), mineral oil was incorporated into the diets on the theory that tansy ragwort alkaloids would be taken up by the oil and passed through the body unchanged and

unabsorbed. Pyrrolizidine alkaloids are soluble in organic solvents at an alkaline pH (Bull et al., 1968).

Dietary treatments were as follows:

1. Positive control: ground corn replacing tansy in basal diet.
2. Negative control: basal diet (10% tansy ragwort).
3. Basal diet with tansy ragwort soaked for 48 hours after being adjusted to pH 8.0 with 4.0 N NaOH.
4. Basal diet with tansy ragwort soaked for 48 hours at pH 8.0, then acidified to pH 4.0 with concentrated HCL.
5. Basal diet + 5% strongly acidic ion exchange resin.
6. Basal diet + 5% strongly basic ion exchange resin.
7. Basal diet + 5% strongly acidic ion exchange resin + 5% strongly basic ion exchange resin.
8. Basal diet + 1% Tris (Hydroxymethyl) Amino methane.
9. Basal diet + 5% strongly acidic ion exchange resin + 5% strongly basic ion exchange resin + 1% Tris (Hydroxymethyl) Aminomethane.
10. Basal diet + 1% mineral oil.
11. Basal diet + 1% mineral oil + 1% Tris (Hydroxymethyl) Aminomethane.
12. Basal diet + 5% strongly acidic ion exchange resin + 5% strongly basic exchange resin + 1% Tris (Hydroxymethyl) Aminomethane + 1% mineral oil.

Third Soaking Experiment

To determine the minimum amount of alkali necessary to detoxify

tansy ragwort and the effect of certain dietary additives, 78 male Long-Evans rats of 60 g initial weight were randomly assigned to each of 13 diets. Animals were individually housed and fed ad libitum. Body weights and feed consumption were measured. The basal diet is listed in Table 9. Dried, ground tansy ragwort was incorporated into all but the control diet at the 15% level. Treatments were prepared as follows:

A. Positive control — Basal diet.

B. Negative control — Basal diet + 15% tansy ragwort, replacing corn.

TABLE 9. BASAL DIET FOR THIRD SOAKING EXPERIMENT

Ingredient	% Diet
Ground corn	49.0
Soybean meal	26.0
Vitamin mix	0.5
Alfalfa (or Tansy ragwort)	15.0
Trace mineralized salt[a]	0.25
Dicalcium Phosphate	0.5
$CaCO_3$	0.8
Corn oil	5.0
Molasses	3.0

a Provides: 8.75 mg Zn, 7.00 mg Mn, 4.38 mg Fe, 0.88 mg Cu, 0.18 mg I, 0.18 mg Co, per kg diet.

C. pH 8.0 Basal diet + 15% tansy ragwort soaked at 8.0 for 48 hours prepared by taking 450 g tansy ragwort: 225 ml NaOH; 1800 ml distilled water.

D. pH 7.5 Basal diet + 15% tansy ragwort soaked at pH 7.5 for 48 hours prepared by taking 450 g tansy ragwort: 214 ml 4N NaOH: 1800 ml distilled water.

E. pH 7.0 Basal diet + 15% tansy ragwort soaked at pH 7.0 for 48 hours prepared by taking 450 g tansy ragwort: 191 ml 4N NaOH: 1800 ml distilled water.

F. pH 6.5 Basal diet + 15% tansy ragwort soaked at pH 6.5 for 48 hours prepared by taking 450 g tansy ragwort: 158 ml 4N NaOH: 1800 ml distilled water.

G. pH 6.0 Basal diet + 15% tansy ragwort soaked at pH 6.0 for 48 hours, prepared by taking 450 g tansy ragwort: 125 ml 4N NaOH: 1800 ml distilled water.

H. 50% of alkali used in pH 6.0 Basal diet + 15% tansy ragwort soaked in half the alkali used to make pH 6.0 for 48 hours, prepared by taking 450 g tansy ragwort: 67.5 ml 4N NaOH: 1800 ml distilled water.

I. Cysteine Basal diet + 15% tansy ragwort + 1% cysteine.

J. Cystine Basal diet + 15% tansy ragwort + 1% cystine.

K. Taurine Basal diet + 15% tansy ragwort + 1% taurine.

L. Cysteine + NaHCO3 Basal diet + 15% tansy ragwort + 1% cysteine + 5% $NaHCO_3$.

M. Barium Basal diet + 15% tansy ragwort + 100 ppm Ba (as

$BaCO_3$).

Due to a possible mineral imbalance from excess sodium, no commercial mineral mixture was used in diets A through F. Instead, calcium and phosphorus were provided as dicalcium phosphate and calcium carbonate. A trace mineralized salt was also added.

Diets C through H included tansy ragwort that had been soaked at different pH's in a range feasible for biological regulation.

Diets I, J, K, and L include sulphur amino acids and a sulphur-containing acid with a sulfonic acid group. Sulfhydryl groups are nucleophilic, and can act as catalysts, promoting reaction with electrophilic pyrroles (Buckmaster et al., 1976). If pyrroles are conjugated with another compound, they will not be available for electrophilic attack of tissue. Taurine may have a sparing effect on cysteine, or may be reactive with pyrroles via the sulfonic acid group.

Barium was included in diet M to assess the suggestion of Pickrell (personal communication) that pyrrolizidine alkaloids may bind to heparin by reaction with its sulfonic acid moiety. Barium precipitates heparin. On this basis, dietary barium could increase the toxicity of tansy ragwort, and implicate inactivation of heparin as one of the sites of toxic action of pyrrolizidines.

Results and Discussion

(a) Rabbit Experiment

Rabbits were highly resistant to ingested tansy ragwort. At the end of the trial, 263 days after experimental feeding had begun, five

of the six rabbits receiving ragwort were alive. The one death was attributed to trauma during heart puncture. Deaths of four of the control rabbits were also due to trauma while taking blood samples.

Tansy ragwort consumption (Table 10) averaged 2335 g or 112.5% of initial body weight. This percentage is double that necessary to kill rats when fed at the 10% level in the diet (Buckmaster et al., 1976). Body weight gain differed between the two groups, most likely due to poor palatability of the tansy ragwort containing diet. Feed consumption was notably lower for the tansy ragwort group.

Blood parameters are listed in Table 11. No difference between control and treatment groups was noted in the albumin/globulin ratio. Rats fed 5% and 10% tansy ragwort had lowered albumin/globulin ratios compared with control animals (Cheeke and Garman, 1974).

Pathology performed on organs removed from the sacraficed rabbits revealed normal gross appearance and only minor microscopic changes. Hepatocytes in all regions of the hepatic lobules appeared swollen and there was a slight distortion of the hepatic cord structure due to swelling. Cytoplasm of the hepatocytes was vacuolated and had a granular appearance. Nuclei were irregular in size and hyperchromatic. There was no evidence of excess fibrosis or bile duct proliferation. No significant changes were noted in the kidney, lung, or myocardium.

This data indicates little effect of dietary tansy ragwort on the rabbit, since all parameters except for some minor liver lesions remained normal over nine months of feeding. However, this was not found to be consistant with results obtained from injecting a crude alkaloid

TABLE 10. RABBIT BODY WEIGHTS AND TANSY RAGWORT INTAKE

	Diet	
	Control	Tansy Ragwort
Initial BW (g)	2114.0 ± 137.0	2101.8 ± 198.0
Final BW (g)	3881.5 ± 55.5	3480.4 ± 99.8
Total gain (g)	1767.5 ± 81.5	1378.6 ± 118.9
Food cons. (g)	30925.0 ± 1819.0	26501.4 ± 2635.0
TR intake (g)	0	2335.6 ± 232.1

TABLE 11. RABBIT BLOOD DATA

Days on Trial	Diet	A/G Ratio	Albumin (g/100 ml)	Ser. Prot. (g/100 ml)
0	Control	0.95	3.79 ± .36	7.76 ± 1.91
	Tansy	1.58	3.73 ± .39	6.09 ± 1.66
10	Control	1.94	3.42 ± .16	5.18 ± 1.00
	Tansy	2.12	3.50 ± .25	5.15 ± 0.97
94	Control	1.66	4.25 ± .15	6.80 ± 1.99
	Tansy	1.15	3.93 ± .20	7.33 ± 1.29
149	Control	2.23	4.12 ± .30	5.96 ± 0.79
	Tansy	1.56	4.18 ± .22	6.85 ± 0.83
263	Control	1.24	3.84 ± .05	6.92 ± 0.12
	Tansy	1.22	3.76 ± .23	6.83 ± 0.50

preparation. After dosing intraperitoneally with 120 mg tansy ragwort alkaloid per kilogram body weight, gross outward appearance of the two rabbits remained normal for eight hours. No further observations were made until twelve hours later when the rabbits were both found dead. Thus, rabbits seem to be somewhat more susceptible to injected tansy ragwort alkaloid than rats. Rats injected with 120 mg alkaloid per kilogram body weight were alive for 24 hours, but began to succumb after 48 hours (Buckmaster et al., 1976).

Susceptibility to injected alkaloid and resistance to ingested tansy ragwort suggested that only the alkaloid that had been injected directly into the peritoneum was reaching the liver. Perhaps alkaloid consumed in the form of tansy ragwort was not being absorbed through the gut wall. The rabbits' gastric and duodenal pH's were not measured, but may be a factor in inhibiting absorption if high (Bull et al., 1968).

Another possible, but as yet untested explanation may be the presence of a pyrrolizidine alkaloid reducing bacteria in the rabbit cecum. The isolation of Peptococcus heliotrinreducans in sheep, a heliotrine reducing bacteria (Lanigan, 1976) presents the possibility of there also being bacteria specific for other pyrrolizidine alkaloids. However, pyrrolizidine alkaloid absorption is thought to be at a point prior to the cecum, although further investigation is necessary. Another fact not supportive of the cecum bacteria theory is that not all monogastric herbivores are resistant to tansy ragwort poisoning. Horses are extremely susceptible (Gilruth, 1903; Van Es et al., 1929). Guinea pigs are susceptible to tansy ragwort alkaloids (Pierson, unpublished data), but

are very resistant to monocrotaline (Chesney and Allen, 1973). Further work is necessary to ascertain the role, if any, of stomach and intestinal microflora in pyrrolizidine alkaloid metabolism.

(b) Mouse Experiment

Mice appear to be less sensitive to tansy ragwort consumption than do rats. Survival time for those mice receiving 10% tansy ragwort was 138 days, and 209 days for those receiving 5% tansy ragwort. Rats live an average of 30 and 52 days with diets containing 10% (Cheeke and Garman, 1974) and 5% (Buckmaster et al., 1976) tansy ragwort, respectively. In an experiment by Hooper (1974), mice consuming 10% tansy ragwort for nine weeks, then 20%, no mortality occurred before day 133. The relatively greater resistance of Hooper's mice may have been due to strain of mouse, potency of the plant material or difference in diet.

Weight gains for the tansy ragwort fed mice were half as much as those receiving alfalfa (Table 12). Since feed consumption was not

TABLE 12. EFFECT OF TANSY RAGWORT CONSUMPTION ON SURVIVAL TIME OF MICE

Treatment	Survival Time (days)	0-75 Days Body Weight Gain (g)
5% Alfalfa	a	10.8 ± 1.6
10% Alfalfa	a	10.8 ± 2.0
5% Tansy Ragwort	208.9 ± 29.5	6.2 ± 2.0
10% Tansy Ragwort	138.0 ± 44.1	2.2 ± 2.4

a Terminated at day 260.

measured, palatability cannot definitely be linked to the difference in body weights.

Considering these results, mice are not as suitable for a susceptible species in pyrrolizidine alkaloid studies as rats, nor are they insensitive enough to be used as a resistant species.

(c) Quail Experiment

Japanese quail were resistant to the effects of ingested tansy ragwort. Birds sacrificed after 252 days of consuming a 10% tansy ragwort diet appeared normal externally. Body weights of control and tansy ragwort-fed groups were similar, being 131 $\pm$ 12 g and 127 $\pm$ 11 g, respectively. Tansy ragwort consumption averaged 422 g per bird. Livers taken from these quail were pale in color. Microscopic lesions varied in severity between birds, the most pronounced being biliary hyperplasia and hepatomegalocytosis. Testicular hyperplasia was found in one male.

Experimental feeding continued until day 365, at which time the remaining birds were sacrificed. Quail were still normal in gross appearance at this time. Data collected from these birds are listed in Table 13. Liver weight, serum protein level, and albumin/globulin ratio differed between control and tansy ragwort-fed groups. The first two parameters were reduced in the tansy ragwort groups, possibly as a reflection of a decreased liver protein synthesis. Pyrroles inhibit cell division due to a drop in DNA-mediated RNA synthesis (McLean, 1970). In rats, the serum albumin level is decreased in pyrrolizidine alkaloid poisoning (Cheeke and Garman, 1974; Buckmaster *et al.*, 1976).

TABLE 13. EFFECT OF TANSY RAGWORT CONSUMPTION ON JAPANESE QUAIL

	Diet	
	Control	Tansy Ragwort
No. of birds	3	7
Final BW (g)	101.5 ± 2.7	108.8 ± 11.9
Liver wt. (g)	5.54 ± 0.58[a]	2.82 ± 0.95[b]
Heart wt. (g)	1.24 ± 0.22	1.19 ± 0.15
Spleen wt. (g)	0.07 ± 0.00	0.06 ± 0.02
Serum protein (g/100 ml)	6.50 ± 0.79[a]	3.28 ± 0.84[b]
Serum albumin (g/100 ml)	1.46 ± 0.23	1.34 ± 0.34
Alb/Glob ratio	0.29 ± 0.01[a]	0.72 ± 0.19[b]

[Means followed by different superscript are significant ($P < .01$)]

However, in quail the total serum protein decreased with little change in serum albumin.

Pathological examination revealed swelling and degeneration of hepatocytes, slight fibrosis, and a tendency for proliferation of small bile ducts in the tansy ragwort-fed quail. Also, hepatic cord structure was distorted, and cell nuclei size varied considerably. In both control and tansy ragwort groups, lung tissue was congested with scattered foci of hemorrhage.

Although these changes are indicative of tansy ragwort poisoning, they were not severe enough to cause death or a change in external ap-

pearance after a period of one year. By day 252, the quail had consumed 5,275% of their initial body weight in tansy ragwort. Amounts equalling 20% of initial body weight are sufficient to cause mortality in rats (Buckmaster et al., 1976).

(d) Non-Ruminant Herbivore Experiment

The variability between species in their reactions to tansy ragwort ingestions was apparent in this experiment. Results have been summarized in Table 14. Of the four species tested, the gerbil is by far the most resistant to pyrrolizidine alkaloid poisoning. No other species in this trial tolerated the 30% tansy ragwort diet for more than two weeks. All gerbils remained normal in external appearance. Feed consumption held at a constant level. Body weights followed a normal pattern (Melby and Altman, 1976). Gerbil survival time averaged 333 days. Necropsy revealed damaged liver, spleen, and kidney tissue. Hepatic megalocytosis, vacuolated nuclei with inclusion bodies, reticulo-endothelial cell hyperplasia and bile duct hyperplasia were in evidence. Spleen tissue was characterized by lymphoid hyperplasia. Multifocal nephritis with tubular dilation was seen in the gerbil kidney. These findings led to a diagnosis of tansy ragwort poisoning. Tansy ragwort intake exceeded 3600% of initial body weight (Table 14).

Several hypotheses to account for the gerbils' resistance to pyrrolizidine alkaloids may be advanced. The gerbil may have pyrrolizidine alkaloid reducing microflora located in the pouches of the stomach.

TABLE 14. SPECIES RESPONSE TO TANSY RAGWORT CONSUMPTION

Species	# of Anim.	Diet	Initial Body Weight (g)	Final Body Weight (g)	Survival Time (day)	Food Consumption (g)	Tansy Ragwort Consumption (g)	TR as % of IBW
Gerbil	5	30%	27.0±2.3	45.6±4.7	333.2±22.5	3262.2±301.6	978.7±90.5	3639.6%
Hamster	10	a	55.8±4.8	142.8±9.0	b	2908.8±265.1	0	0
Hamster	10	10%	51.2±5.2	68.3±11.3	158.5±53.5	1729.2±724.3	172.9±72.4	337.7%
Guinea Pig	4	10%	196.5±19.2	229.8±19.2	117.3±26.8	2062.8±620.4	233.5±63.1	118.8%
Rat	9	30%	73.3±5.1	55.1±3.0	13.4±1.9	154.3±40.1	46.3±12.0	63.3%

a Control diet.

b Terminated at day 224 when all hamsters on 10% tansy ragwort diet had died.

If so, the alkaloids would be destroyed before reaching the area of absorption further along the digestive tract. Detoxication of alkaloid in the liver is another possibility. No information is available on hepatic pyrrole production rates in this species.

Table 15 lists post-injection survival times for all species tested. Gerbils were resistant to both monocrotaline and jacobine. However, injection of the composite of tansy ragwort alkaloids caused mortality. This indicates that an alkaloid(s) in this preparation, other than jacobine, was toxic to gerbils. The specificity and location of detoxication mechanisms remain to be clarified. The resistance to toxic effects of monocrotaline and jacobine must stem from a specific detoxifying mechanism acting post-absorptively. Contrastly, susceptibility to injected tansy ragwort alkaloid and resistance to ingested tansy ragwort, point toward the reducing microorganism theory or a lowered absorption of these alkaloids.

Hamsters proved to be somewhat resistant to both ingested and injected tansy ragwort alkaloid. Death from ingestion of the plant occurred after an average of 159 days. Total tansy ragwort consumption was 173 g or 338% of initial body weight. Growth rate of the hamsters receiving 10% tansy ragwort in the diet was less than that of control animals.

Pathological examination of this species did not reveal the severe lesions usually associated with chronic tansy ragwort toxicity. Only mild liver degeneration was observed. In some animals, hepatocytes with vacuolated nuclei were noted. In one animal, a darkened liver was found

TABLE 15. INJECTION OF MONOCROTALINE (M), JACOBINE (J), AND A COMPOSITE OF TANSY RAGWORT (T) ALKALOIDS INTO VARIOUS SPECIES[a]

Species	M (mg/kg)	Survival Time (days)	J (mg/kg)	Survival Time (days)	T (mg/kg)	Survival Time (days)
Rat	150	b	150	b	150	2
	150	2	200	2		
Hamster	150	5	125	4	125	5
Guinea Pig	500	b	50	b	50	b
			100	3	100	3
			150	1	150	3
Gerbil	100	b	100	b	100	b
	150	b	150	b	150	2
	250	b	200	b	200	1

a One animal per dosage level.

b No mortality within one week following injection.

in association with scattered hemosiderin deposits. Red blood cell destruction may have been taking place with excess iron being temporarily stored in the liver as hemosiderin.

Injected hamsters died from each alkaloid. Survival times on medium level doses were longer than for the other species.

Hamsters have an extremely high rate of *in vitro* pyrrole production (Shull *et al.*, 1976 b), and would therefore be expected to be very susceptible to tansy ragwort poisoning. This was not the case in this trial, nor were the histological findings indicative of severe pyrrolizidine alkaloid toxicity. They are similar to the rabbit in this respect. Further investigation of the mechanism of resistance of these animals to pyrrolizidine alkaloids would be useful.

Unlike their total resistance to the toxic effects of monocrotaline (Chesney and Allen, 1973) and moderate resistance to retrorsine (White *et al.*, 1973) and jacobine (Hopkirk and Cunningham, 1936), guinea pigs were found to be quite susceptible to the alkaloids in tansy ragwort. Death occurred after 117 days of consuming a diet containing 10% tansy ragwort. Total tansy ragwort intake equalled 119% of initial body weight.

Classic symptoms of tansy ragwort poisoning were found upon microscopic examination of internal organs. The mottled liver section revealed hepatic necrosis with fibrosis and biliary hyperplasia. Megalocytosis and vacuolated nuclei with inclusion bodies were present. Large amounts of hemosiderin indicating red blood cell breakdown were found.

In agreement with results of Chesney and Allen (1973), guinea pigs were resistant to a massive dose of injected monocrotaline. However, both jacobine and the composite tansy ragwort alkaloid preparation caused death within three days at moderate dosage levels. Hopkirk and Cunningham (1936) injected 380 mg of jacobine/kg into guinea pigs. Necropsy revealed definite changes in liver tissue in the form of fibrosis and bile duct proliferation.

The guinea pig's detoxication must be specific for particular alkaloids, since monocrotaline has little effect in this species, but some or all of the alkaloids in tansy ragwort cause fatal liver necrosis. A pyrrolizidine alkaloid reducing microorganism is probably not the mechanism by which monocrotaline is detoxified in this species. Chesney and Allen (1973) suggest an increased activity of the N-oxide forming enzyme system in the guinea pig. A high level of non-toxic N-oxide metabolites is found after injection of monocrotaline.

The rats in this experiment may have died of malnutrition rather than tansy ragwort poisoning. Feed consumption per day was very low, averaging about 11 g per animal. The 30% tansy ragwort diet was extremely unpalatable to these rats. All had diarrhea and were hyperirritable and aggressive. Survival time averaged 13 days with rats consuming 46 g of tansy ragwort or 63% of inital body weight. This amount of tansy ragwort will cause death in rats receiving it as a lesser percentage of their diet. However, the afore-mentioned symptoms and normal appearing livers point toward malnutrition as the cause of death.

Thirty percent tansy ragwort was incorporated in the diet in an attempt to reduce the length of feeding trials in pyrrolizidine alkaloid studies. A compromising level of tansy ragwort, somewhere between ten percent and thirty percent will have to be established in future trials in order to lessen survival time and still maintain an adequate feed intake.

(e) First Soaking Experiment

The results of this feeding trial are summarized in Table 16. Nine of the ten rats in the pH 8.0 group were still alive when experimental feeding was terminated at 185 days. The one death was probably due to an illness other than tansy ragwort poisoning. Feed consumption for this animal was low for several months before death. Rats dying from tansy ragwort poisoning ate normally until the last few days of life. Feed consumption and average daily gain for the pH 8.0 treatment approached that of the control animals. Palatability was good with this NaOH soaked treatment.

As measured by survival times, soaking tansy ragwort at pH 8.0 was most effective in reducing its toxicity, followed by McDougall's Nutrient Buffer, pH 7.0, autoclaved cattle rumen fluid, and active cattle rumen fluid. Tansy ragwort soaked at pH 4.0 showed no difference from the untreated tansy ragwort-containing diet. Average total tansy ragwort consumed by the pH 8.0 group was 234.5% of their initial body weight. Tansy ragwort equalling 82.3% of initial body weight killed all animals in the untreated group.

Rats on the McDougall's Nutrient Buffer diet survived longer than

TABLE 16. EFFECT OF ALKALI AND RUMEN FLUID TREATMENT ON TANSY RAGWORT FED TO RATS

Treatment	Init. BW (g)	Final Bw (g)	Avg. Daily Gain (g) day 0-30	Surv. T. (day)	TR Cons. (g)
Control	67.0±6.0	476.4±68.7	5.8 ± 0.6^{d}	a	0
pH 4.0	66.3±6.0	201.7±51.5	3.3 ± 0.3^{eg}	83.0 ± 31.9^{df}	60.8 ± 28.8^{dfg}
pH 8.0	73.7±4.0	420.1±88.1	5.1 ± 0.7^{d}	b	172.8 ± 34.8^{e}
pH 7.0	73.1±5.0	294.8±24.1	4.3 ± 0.5^{fg}	125.7 ± 21.6^{ef}	101.1 ± 23.8^{dg}
Untreated TR	70.4±3.5	212.7±31.1	3.4 ± 0.5^{eg}	82.9 ± 21.0^{df}	58.0 ± 19.9^{fg}
McDougall's	67.7±6.0	228.5±56.1	3.4 ± 0.5^{eg}	c	94.1 ± 40.7^{g}
Active CRF	70.8±5.2	241.5±61.4	3.3 ± 0.5^{eg}	105.4 ± 28.4^{f}	75.6 ± 22.8^{g}
Autocl. CRF	65.9±5.5	259.8±56.1	4.0 ± 0.3^{g}	123.7 ± 39.0^{ef}	99.4 ± 42.9^{g}

a Terminated at 142 days.

b Terminated at 185 days, which is significantly ($P<.01$) longer than any of the survival times.

c Terminated at 185 days, which is significantly ($P<.01$) longer than any of the survival times.

Means followed by different superscripts are significantly different ($P<.01$).

all treatments except pH 8.0 and control. Two animals were still alive at the 185 day termination date. Feed consumption and average daily gain were low, possibly due to the buffer being unpalatable. However, the buffer appears to have kept the pH high enough for degradation to occur while soaking.

Among the pyrrolizidine alkaloids, a huge variability is observed in stability under alkaline conditions (Bull et al., 1968). Soaking in 0.5N NaOH and water at room temperature causes alkaloid decomposition (hydrolysis). The moieties thus formed do not cause the tissue damage that the parental alkaloid may cause to develop. It may be of interest to identify the breakdown products of the alkaloids in tansy ragwort with paper chromatography.

This pH effect theory is also reflected by the cattle rumen fluid treatments. The active cattle rumen fluid produced acid during incubation, lowering the pH. Alkaloid remained unchanged in the acid environment and was therefore still toxic. On the other hand, the autoclaved cattle rumen fluid had no working bacteria for acid production and so remained at a higher pH, suitable for alkaloid degradation. Soaking at pH 8.0, however, produced the best protective effect. The higher the pH, the more decomposition to non-toxic products must occur, as long as an imbalance due to too much alkali is not brought about.

(f) Second Soaking Experiment

In order to simulate field conditions more closely, a natural ingredient diet containing ground corn, soybean meal, and molasses was formulated to replace the casein based diet previously used. The new

TABLE 17. EFFECT OF VARIOUS DIETARY ADDITIVES ON RATS FED 15% TANSY RAGWORT

Treatment	Init. BW (g)	Final BW (g)	Surv. T. (day)	Food Cons. (g)	TR Cons. (g)
Control	64.6±4.9	409.4±42.7	a	2106.4±158.2	0
Untreated TR	64.6±3.7	107.0±10.4	53.2±5.5	619.0±87.9	61.9±8.8[c]
Alkali	63.2±6.4	124.0±69.6	48.8±43.1	716.2±681.6	71.6±68.2[c]
Re-Acidified	64.0±6.1	155.2±21.7	77.4±21.9	1102.6±345.1	110.3±34.5[d]
IER-SA	64.6±2.3	94.6±27.8	50.0±10.4	589.2±173.9	58.9±17.4[c]
IER-SB	59.4±3.3	93.4±12.9	52.4±8.8	576.8±296.8	57.6±29.7[c]
IER-Sa+SB	65.2±7.6	95.8±15.1	57.2±11.1	647.0±191.5	64.7±19.1[c]
Tris	59.8±6.9	98.8±13.1	44.0±5.9	499.4±93.2	49.9±9.3[c]
IER-SA+SB, Tris	60.2±4.5	90.2±14.4	54.2±1.9	624.2±40.7	62.4±4.1[c]
Mineral Oil	63.0±3.9	110.8±31.8	49.4±12.5	586.6±182.4	58.6±18.2[c]
Tris+Min. oil	66.0±5.6	96.7±19.9	40.0±1.9	475.0±54.1	47.5±5.4[c]
Combination[b]	63.8±5.2	72.6±8.9	38.8±11.9	438.4±131.5	43.8±13.1[c]

a Control rats were terminated at 101 days.
b Combination = 5% strongly acidic ion exchange resin (IER-SA); 5% strongly basic ion exchange (IER-SB) resin, 1% Tris, and 1% mineral oil.
Means followed by different superscript are significant ($P<.05$).

diet, however, may have imposed problems.

Treating tansy ragwort by soaking at pH 8.0; previously very successful in reducing toxicity, showed no effect of detoxication (Table 17). However, survival times of the individual animals on this diet reveal that three of the five rats died within three weeks, while the other two animals lived 81 and 118 days. This variation may indicate a possible mineral imbalance in the short-lived rats, imposed by ingredients in the diet. The soaking solution for this treatment was made alkaline with 4N NaOH. This, in combination with a commercial mineral mixture may have increased the sodium level in the body to a point of imbalance.

Excess sodium must be removed from the body fluids by the kidneys (Guyton, 1971). Under normal conditions, sodium is reabsorbed from the glomerular filtrate to retain the proper cation concentrations. When a sodium ion is reabsorbed, electro-negativity will result, causing a negatively charged chloride ion to follow across the tubular membrane. However, with an excess of sodium ions, few will be reabsorbed, and therefore most chloride ions will also remain in the filtrate. This results in excessive loss of chloride ions to the urine. The balance of chloride ions and bicarbonate ions is important in the body's acid-base balance.

Necropsy of one of the rats dying within three weeks of first consuming the alkaline (pH 8.0) tansy ragwort diet revealed liver biliary hyperplasia. Since biliary hyperplasia is one of the histological changes associated with tansy ragwort toxicity, perhaps pyrrolizidine

alkaloid poisoning should be considered as a possible cause of death in combination with a mineral imbalance.

The treatment that had been re-acidified to pH 4.0 after soaking at pH 8.0, probably did not have this mineral imbalance due to chloride ions being replaced in the system with the addition of HCl. This maintenance of metabolic homeostasis is reflected in the length of survival time for this group of 77 days. The inactivation of pyrrolizidine alkaloids under alkaline conditions is apparently irreversible, since re-acidification (treatment 4) did not restore its toxicity.

The addition of 1% mineral oil had no effect on the survival time of animals receiving it as a part of their tansy ragwort diet. The toxic effects of mineral oil [decreasing absorption of fat soluble vitamins and impairing utilization of nutrients (Ershoff and Hernandez, 1958)] outweighed any theoretical beneficial effect of binding the pyrrolizidine alkaloids and rendering them unabsorbable throughout the digestive tract.

One percent Tris (Hydroxymethyl) Aminomethane in dry form apparently either could not exert enough buffering capacity in the gastrointestinal tract to allow degradation of tansy ragwort alkaloids, or digesta was not given sufficient time at the higher pH for decomposition to occur. In the previous experiment, soaking was carried out for 48 hours, and prior to incorporation into the diet. All diets containing Tris (Hydroxymethyl) Aminomethane resulted in survival times shorter than that of untreated tansy ragwort.

Neither form of ion exchange resin had a significant effect on

any of the parameters measured. Strongly basic ion exchange resin, as in the case with Tris (Hydroxymethyl) Aminomethane, either did not raise the gastric pH high enough or did not allow enough soaking time. The strongly acidic ion exchange resin may have preferentially bound ions other than those formed from the pyrrolizidine alkaloids, since survival time was similar to that of untreated tansy ragwort.

(g) Third Soaking Experiment

Soaking tansy ragwort in alkali provided protection against the toxic effects of pyrrolizidine alkaloids in a natural ingredient diet, while sulfur containing amino acids did not. Results are listed in Table 18.

Tansy ragwort that had been soaked at a pH of 7.5 appeared to be most effective in reducing toxicity. A pH of 8.0 may be likely to create problems with mineral imbalance. Four of six animals within this treatment died in less than two months, the other two living five and eight months. As in the Second Soaking Experiment, a detrimental sodium-chloride interaction is indicated. The pH's lower than 7.5 resulted in improved survival times over that of untreated tansy ragwort, but to a lesser degree.

Using 7.5 as an optimum pH, minimum soaking time should be determined. In practical terms, digesta turnover time in the bovine rumen is 50-60 hours (Church, 1975). Although this seems like abundant time for detoxication to occur if a pH of 7.5 could be established, consideration must be given to the possibility of alkaloids leaching out of the plant material soon after reaching the rumen. In this case, mini-

TABLE 18. EFFECT OF ALKALI AND VARIOUS ADDITIVES ON RATS FED 15% TANSY RAGWORT

Treatment	Init. BW (g)	Final BW (g)	Surv. T. (days)	Food Cons. (g)	TR Cons. (g)
Control	90.3±4.2	427.1±51.6	a	2516.4±228.3	0
Untreated TR	90.7±4.2	112.4±17.9	50.6±9.8[f]	878.6±220.5	133.7±33.1[d]
pH 8.0	90.5±5.4	181.0±96.8	87.5±82.8	1744.4±2055.4	261.7±308.3
pH 7.5	91.0±5.1	242.8±71.4	151.7±59.8[e]	2262.0±921.6	339.3±138.2[c]
pH 7.0	91.0±3.7	162.7±34.5	64.2±18.2[f]	1119.0±329.9	167.9±49.5[d]
pH 6.5	90.8±4.2	143.0±32.0	95.8±49.3	1712.2±1179.4	231.8±144.1
pH 6.0	90.5±5.4	198.5±68.6	105.7±73.5	1708.3±1191.7	256.3±178.8
pH half of 6[b]	89.8±5.4	157.8±49.5	71.5±38.0[f]	1156.0±629.0	173.4±94.4[d]
1% cysteine	90.0±5.9	112.8±18.0	47.3±3.1[f]	775.7±124.8	116.4±18.7[d]
1% cystine	89.7±6.3	105.3±14.4	46.7±6.5[f]	787.3±57.0	118.1±8.5[d]
1% taurine	89.5±5.0	106.8±22.7	47.4±4.6[f]	818.2±217.6	122.7±32.6[d]
1% cysteine + 5% $NaHCO_3$	90.8±8.1	109.3±14.6	48.2±8.5[f]	709.3±162.6	106.4±24.4[d]
110 ppm Ba	89.8±6.4	111.3±16.6	43.7±2.5[f]	629.7±84.4	94.5±12.7[d]

a terminated at 233 days when last tansy ragwort-fed rat had died.
b half the amount of 4N NaOH necessary to produce pH 6.0.
Means followed by c or d are significantly different ($P<.05$).
Means followed by e or f are significantly different ($P<.01$).

mum soaking time would be important.

The diets providing nucleophilic sulphydryl groups (I, J, K, and L) had no effect in protecting nucleophilic tissue from attack by pyrrolic metabolites. This is exemplified by survival times and tansy ragwort consumption in amounts similar to that of the untreated tansy ragwort group. Cysteine has been previously successful in providing protection against tansy ragwort poisoning in a casein based diet (Buckmaster et al., 1976). Clarification of the conflicting responses to cysteine and other sulfhydryl donors with purified vs. natural ingredient diets requires further study. Obviously a protective effect of cysteine would only be of significance in diets based on natural ingredients.

The addition of barium slightly reduced survival time and amount of tansy ragwort necessary to cause mortality. Barium causes heparin, a sulfated compound in the blood, to precipitate. Pickrell (1977, personal communication) has suggested that intravascular coagulation in pyrrolizidine poisoning might be explained by a pyrrolizidine-heparin reaction, and that barium would increase the bound heparin. The results are supportive of that hypothesis.

IV. SUMMARY

As summarized in Table 19, species response to the pyrrolizidine alkaloids in tansy ragwort is quite varied. Of the seven species tested, the Japanese quail and gerbil appear to be most resistant.

When seeking a laboratory animal model for larger species, perhaps the gerbil possesses more appropriate qualities than the Japanese quail. Gerbils are mammalian, monogastric herbivores, and harbor microorganisms in the pouches of their stomachs which may closer simulate conditions found in ruminants and horses. The high percentage of tansy ragwort in the diet that the gerbil will tolerate may also be of advantage.

As in the past, the rat will be continued to be used as a susceptible species. The quantity of tansy ragwort necessary to cause death is low, and survival time is short. The size of the rat and ease of care are also important factors.

Alkali treatment of tansy ragwort appears to detoxify the alkaloids to an extent that is worth pursuing. Although survival time results were more promising with a casein based diet than a natural ingredient diet, the latter is the only practical course to follow. Survival times were increased from 51 days with untreated tansy to 152 days with alkali treatment.

Although used with previous success, sulfur amino acid additives in a casein based diet (Buckmaster et al., 1976) had no effect on increasing survival time in natural ingredient diets. Soaking tansy ragwort in cattle rumen fluid and McDougall's Nutrient Buffer increased survival times

TABLE 19. SPECIES RESPONSE SUMMARY

Species	Lethal Intake of TR as a % of Initial Body Weight	Response to Injected TR Alkaloids		*In vitro* Liver Pyrrole Production Rate
Gerbil	3639%	150 mg/kg	death in 48 hrs	e
Guinea pig	119%	100 mg/kg	death in 72 hrs	e
Hamster	338%	125 mg/kg	death in 120 hrs	very high[g]
Japanese quail	5275%[a]	115 mg/kg	LD_{50}[d]	very low[g]
Mouse	b	e		high[g]
Rabbit	113%[c]	120 mg/kg	death in 24 hrs	very high[g]
Rat	63%	120 mg/kg	death in 48 hrs[f]	high[g]

a When terminated at 252 days, birds had consumed tansy ragwort equalling 5275% of initial body weight.
b Feed consumption not measured.
c When terminated at 263 days, animals had consumed tansy ragwort equalling 113% of initial body weight.
d Buckmaster *et al.* (1977).
e No data available.
f Buckmaster *et al.* (1976).
g Shull *et al.* (1976).

for rats receiving tansy ragwort as 5% of their casein-based diet.

V. CONCLUSIONS

Progress has been made toward a practical solution to the widespread tansy ragwort toxicity problem. From this investigation, it seems that soaking in a solution of elevated pH will allow detoxication of tansy ragwort. This concept may be plausible in practical terms if an appropriate buffer which will raise gastro-intestinal pH's to 7.5, with no deleterious side effects can be found. Cattle and horses exposed to tansy ragwort could be offered such buffers through a mineral block or liquid feed supplement dispenser.

Movement of digesta through the equine stomach is rapid, therefore necessitating an elevated pH for the short time while tansy ragwort is present. A buffer taken with the feed might raise the gastric pH sufficiently, then aid in sustaining the already higher intestinal pH to allow maximum time for alkaloid detoxication. Further investigation into minimum time necessary for detoxication to occur may prove valuable.

Possible interaction between pH changes and bacterial microflora populations should also be examined. If pyrrolizidine alkaloid reducing bacteria exist for the alkaloids in tansy ragwort, the pH which they find optimum should also be considered. Since the gerbil is known to be resistant, microorganisms in its stomach pouches should be identified as to their affinity for tansy ragwort alkaloids. Changing the pH of the gerbil stomach through the use of buffers may clarify any interactions and lead to further progress toward field applications.

Determination of the biochemical mechanisms by which some animals, such as the gerbil, rabbit, and Japanese quail, are resistant to dietary tansy ragwort may aid in the identification of ways to increase the resistance of livestock to the toxic effects of this plant.

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