A Study on the Toxicity of Sesbania Drummondii in Chickens and Rats.

Marie-lorraine Marceau-day

Louisiana State University and Agricultural & Mechanical College

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A study on the toxicity of *Sesbania drummondii* in chickens and rats

Marceau-Day, Marie-Lorraine, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1988
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UMI
A STUDY ON THE TOXICITY OF *SESBANIA DRUMMONDII* IN CHICKENS AND RATS

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in

The Department of Veterinary Medical Sciences

by

Marie-Lorraine Marceau-Day
BSc. (Agr), McGill University, 1974
May, 1988
DEDICATION

For my children, Carolyn Anne and Benjamin Marc with love. You have been my inspiration. Thank you for your encouragement and patience. This work is also dedicated to my husband, Dr. Donal F. Day for his understanding, reassurance and commitment. It has been an arduous task but a new day dawns brightly.
ACKNOWLEDGEMENTS

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ABSTRACT

A comparative study of two susceptible species, the chicken and the rat, to crude and partially purified extracts of the plant *Sesbania drummondii* was undertaken. Partial physical and chemical characterization of crude sesbania extracts has been accomplished including temperature and pH stability, molecular weight fractionation and high pressure liquid chromatography and thin layer chromatography retention times. The toxicity of these different fractions could be differentiated with respect to weight loss, mortality, morbidity, serum protein changes and effect on PChE (Pseudocholinesterase) activities. The results indicated that more than one toxin exists in crude extracts of this plant. The pathophysiological findings could not be attributed to the presence of sesbanimide as the only major toxin of sesbania. The effects of sesbania extracts on serum protein concentration and PChE activity were similar but not identical to the effects of the protein synthesis inhibitor, cycloheximide. Additional qualitative and quantitative differences between the effects of sesbania extracts and cycloheximide were noted following histological and electron microscopic examination. The results suggested that inhibition of protein synthesis is not the only mechanism of sesbania intoxication. Some evidence was obtained to indicate that inhibition of export protein release from the liver occurred during sesbania intoxication. Further investigation suggested that the liver is a major target organ, though not the sole target organ, in an experimentally induced episode of sesbania intoxication. Phenobarbital gave partial protection against sesbania-induced weight loss and morbidity in the adult chicken but failed to protect the immature birds. No protective influence was noted for phenobarbital pretreatment in the sesbania treated rat. Evidence was obtained for the presence of several new microsomal glutathione-S-transferase isozymes which could be modified by sesbania treatment. Glutathione peroxidase activities were depressed by sesbania treatment. The induction of the cytochrome P-450 monooxygenase system by sesbania treatment in the chicken but not the rat suggested further species differences. The induction of the microsomal monooxygenase system and glutathione-S-transferases suggested the possibility of the release of free radicals or strong electrophiles by crude extracts of sesbania.
GOALS AND RATIONALE

Intoxication of cattle by *Sesbania drummondii* is a frequent and serious problem in Louisiana. It has been previously shown that most animal species are susceptible to the actions of this plant although there may be quantitative differences in response. A comparison of two susceptible species (chickens and rats) will be undertaken to evaluate differences in response between avian and mammalian species.

This study has two major postulates. The first is that sesbania induced toxicity is the result of the presence of multiple toxins. The experimental approach to this problem will include a description of some of the physico-chemical properties of the toxic principles. These physical properties will include the effect of temperature (both freezing and heating), pH stability and its effect on weight loss, serum protein concentration and PChE (Pseudocholinesterase) activity. Since *Sesbania drummondii* is a leguminous plant and several lectins have been isolated from this family of plants, studies to detect the presence of a lectin and the interaction of sesbania extracts with a lectin (Concanavalin-A) as well as its interaction with reactive carbohydrates (Sephadex™ G-25) will be examined. The toxic compounds will be separated using hollow fiber filtration and the determination of molecular weight will be done by ultrafiltration. The partition of toxic signs including weight loss, decreases in serum protein concentration and decreases in PChE...
activity should help to determine the presence of multiple toxins in crude sesbania extracts.

Sesbanimide, a cytotoxic compound, has been previously isolated from the seeds of *Sesbania drummondii* (Powell et al. 1983, 1984). It possesses a structural similarity to the glutaride class of antibiotics which are exemplified by cycloheximide. Cycloheximide produces its toxicity by inhibiting the synthesis of proteins. Comparison studies to test the similarities between cycloheximide and sesbania intoxication will be undertaken. These studies will be extended to include a histologic and electron microscopic evaluation of the effects of sesbania and cycloheximide in the liver in an effort to determine similarities in mechanism of action.

Flory and Hébert (1984) first reported that sesbania treatment dramatically depressed the concentration of circulating serum proteins in chickens. Based on the knowledge that most serum proteins are export protein products from the liver, it is further postulated that the liver represents a major target for sesbania intoxication. It will be important to determine whether the decrease in serum protein concentration is the result of the inhibition of protein synthesis or whether this decrease reflects an inability to release serum proteins (export proteins) from the liver after they are synthesized. This problem will be addressed by comparing the activities of selected marker enzymes in both the serum and liver fractions from control and sesbania treated rats and chickens.

Since the liver is postulated to be a site of sesbania action, the effects of sesbania on this organ will be assessed in different liver
fractions. The liver will be fractionated into microsomal and cytosolic portions by differential centrifugation. A select cross-representative group of enzyme activities will then be determined to attempt to evaluate the mechanism of action of sesbania toxins in this organ.

The work will lead to a better understanding of the mechanisms of sesbania-induced toxicity. The work is fundamental to the future development of therapeutic intervention in accidental sesbania intoxication. No specific treatments are currently available for the management of sesbania intoxication.
REVIEW OF LITERATURE

Introduction:

Sesbania drummondii is a small perennial toxic shrub or tree that grows to an average height of 1.8 to 2.7 meters. It is commonly found throughout the Southeastern United States, from Florida to Texas. Its normal habitats are wet, marshy soils and sandy loams where it becomes well-established. Other habitats include low wooded areas, the edges of bayous, marshland, road sides, railroad embankments, the edges of ponds, ditches, prairies and sandy beaches (Lasseigne, 1973 and Marshall et al, 1985).

The literature is inconsistent with respect to the correct botanical classification of this plant. It has been variously identified and reported as Sesbania drummondii, Daubentonia drummondii, Daubentonia longifolia and Sesbania cavellesii. It possesses a considerable taxonomic similarity to Glottidium vesicarium (Broughton and Hardy, 1939; Correll and Johnson, 1970; Duncan et al, 1955; Emmel, 1935 and Emmel, 1944). Several trivial names for this plant have come into common usage including coffee bean (Marsh, 1920), bagpod (Emmel, 1944 and Robey, 1925), coffee senna (Featherly et al, 1943) and bladderpod (Newberne, 1953).

The leaves of Sesbania drummondii are alternate and pinnate with several leaflets (usually 12-20). Yellow flowers, which are frequently tinged with purple, are present in axial racemes. The fruit of the plant is many-seeded, linear and compressed into a bladder-shaped pod. The shape of the fruit is the source of one of
its trivial names, bladderpod. Some 30 species of the genus are recognized in tropical and subtropical regions (Lasseigne, 1973), but not all species are poisonous (Kingsbury, 1964). The genus name is derived from one of the species said to be sesban. The seeds of *Sesbania drummondii* are toxic to all species of livestock, domestic fowl and humans (Ellis, 1975).

**Habitat and Identification:**

*Sesbania drummondii* was first introduced into Florida from the West Indies as an ornamental (Lasseigne, 1973). *Sesbania drummondii* became rapidly established and soon spread westward into Texas and northward into the Carolinas. By 1941, it was detected as far northwest as central Oklahoma (Lasseigne, 1973). This rapid expansion increased the potential for livestock poisoning. As late as 1973, *Sesbania drummondii* was confined to the southern half of the state of Louisiana (Lasseigne, 1973). This distribution corresponded to the coastal wetlands and coastal plains. Today, it can be readily isolated from most areas of the state including the alluvial plains which were devoid of *Sesbania drummondii* at the time of the 1973 study.

The mature pods remain on the plant long after most of the surrounding foliage has died from frost. In areas where food is scarce or where livestock are left to forage on their own, sesbania may be the only abundantly available plant during winter (Duncan et al., 1955). In addition, cattle, in particular, may develop an appetite for sesbania. This attraction to sesbania plants may persist long after hay or grass is offered (Emmel, 1944).
Most of the toxicity of the plant is associated with the mature pods (containing the seeds, Terblanche et al., 1966). The fresh leaves are much less toxic and the pods (minus the seeds) display little if any toxic activity (Marsh, 1920; Marsh and Clawson, 1920; Simpson and West, 1953 and Terblanche et al., 1966). Although cattle are now the most frequently afflicted species, sesbania toxicity was originally observed in a herd of goats (Marsh, 1920).

Classification:

There is little taxonomic evidence to support the classification of Sesbania, Daubentonia and Glottidium as separate genera in the legume family. Their classification should be as a species of Sesbania (Lasseigne, 1973). Despite morphological pod and bloom differences, chromosome numbers and pairing appear to be identical in all these genera (Kingsbury, 1964). The Daubentonia drummondii of Rydb, Daubentonia longifolia of Cocks and the Sesbania drummondii of Turner are considered to be a single distinct species (Lasseigne, 1973). For the purposes of this study, the classification put forth by Kingsbury (1964) has been adopted. The complete classification is shown in Table 1.

A related plant, Sesbania punicea, a native of South America, is establishing itself within the same distribution area as the Sesbania drummondii species (Lasseigne, 1973). This research has dealt with the effects of the drummondii species and not the closely related punicea. The former species has yellow blooms while those of punicea are orange-red. Nevertheless, both species appear to produce identical clinical signs and it would be difficult
**TABLE 1**

Classification of *Sesbania drummondii* according to Kingsbury (1964).

<table>
<thead>
<tr>
<th>Kingdom:</th>
<th>Plantae</th>
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<tr>
<td>Phylum:</td>
<td>Spermatophyta</td>
</tr>
<tr>
<td>Class:</td>
<td>Angiospermae</td>
</tr>
<tr>
<td>Subclass:</td>
<td>Dicotyledonae</td>
</tr>
<tr>
<td>Order:</td>
<td>Rosales</td>
</tr>
<tr>
<td>Family:</td>
<td>Leguminosae</td>
</tr>
<tr>
<td>Genus:</td>
<td><em>Sesbania</em> (Daubentonia)</td>
</tr>
<tr>
<td>Species:</td>
<td><em>drummondii</em></td>
</tr>
</tbody>
</table>
to differentiate between the two species during an episode of poisoning (Correl and Johnson, 1970).

Pathophysiology:

Many animal species appear to be susceptible to the actions of sesbania (Terblanche et al., 1966). The effects of *S. punicea* are included because the pathophysiology of the two species appears identical. Their botanical similarity strongly suggests that both species should contain the same or similar toxins. A potential toxin, sesbanimide, has been independently isolated from both species (Gorst-Allman et al., 1983 and Powell, 1983; Powell et al., 1983). It has been postulated that this may be the major toxin in this genera (Powell, personal communication).

Instances of range poisoning by these plants have been recognized since the latter part of the 19th century. The first thorough investigation of an intoxication by *Sesbania drummondii* was undertaken by Marsh and Clawson (1920). The incident involved the poisoning of sheep which had been travelling to a fresh pasture in Texas. The plant was abundant along the road sides and there was evidence of foraging by the herd which resulted in substantial livestock losses.

Sheep poisoned by sesbania display an assortment of pathological signs. The initial signs were a general restlessness and depression. This was followed by kyphosis, anorexia and diarrhea. Prior to death, the animals had shallow, rapid breathing, a weak and rapid pulse and became comatose. Post-mortem examination revealed the presence of tarry blood and / or multiple
petechial hemorrhages of the gastrointestinal tract. Histological examinations showed the presence of degenerative changes in the gastric and mesenteric lymph nodes. A severe gastric enteritis was observed clinically. In sheep, this gastric enteritis was confined to an area posterior to the rumen (Broughton and Hardy, 1939).

Several experimental intoxications of cattle have been produced with sesbania (Emmel, 1935; Featherly et al., 1943; Newberne, 1953; Simpson and West, 1953). Cattle dosed at 2% of body weight exhibited signs within the first 24 hours and death within 48 hours after a single dose. With chronic low doses, death occurred after 2 weeks at a daily dose of 0.15% of body weight. The toxic effects of sesbania poisoning appeared to be cumulative (Broughton and Hardy, 1939; Emmel, 1935; Emmel, 1944 and Shealy and Thomas, 1928). Poisoned cattle occasionally displayed constipation but their usual response is similar to other species (i.e. diarrhea). Consumption of the seeds of *Sesbania drummondii* or other closely related species produced hemorrhagic diarrhea. Post-mortem examination showed severe hemorrhagic inflammation of the small intestine and abomasum. Generalized albuminous degeneration of the kidneys and liver degeneration was noted histologically by Featherly *et al.* (1943). This was frequently accompanied by limited necrosis of the liver and complete rumen stasis. The rumen very often contained sprouted seeds. Changes in the lymphatic system and smooth musculature were also noted in poisoned cattle. The presence of small thrombi of degenerated red blood cells, throughout the circulatory system, were also reported
Small quantities of the seeds produced diarrhea and tachycardia in cattle, sheep and goats (Emmel, 1944). Clinical biochemical analyses showed an increase in blood urea nitrogen (BUN), creatinine, glucose, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) in these animals. Necropsy findings in cattle were similar to those observed in sheep (Terblanche et al. 1966). As with many toxicoses, the severity of the observed signs was dose-related (Marsh, 1920).

Poisoning of fowl by sesbania has been predominantly experimental. Duncan et al (1955) fed seeds of the closely related *Glottidium vesicarium* (*Sesbania vesicarium*) to domestic fowl at rates ranging from 1.13 to 2.00% of the body weight. This range was found to be lethal. Signs of intoxication were diarrhea and prostration. Post-mortem examination showed the presence of dark tarry blood and a dark congested comb in the males. Necrotic enteritis, severe inflammation and necrosis of the gizzard were found (Duncan et al., 1955). Duncan also reported fatty degeneration of the liver and edema of most of the parenchymal organs of the body. Signs first appeared 8 to 30 hours post exposure and were similar to those described for poisoned sheep. In a similar experiment with *Sesbania drummondii*, Duncan et al. (1955) found evidence for acute toxicity in dosed fowl. All birds exhibited signs of depression. At higher doses, this depression was accompanied by decreased respiratory rates and lowered body temperatures. These symptoms were followed by the production of a mucoid diarrhea, dypsnea, anorexia, and a general muscular weakness. The birds were
prostrate until death, which was immediately preceded by mild convulsions, muscular tremors and vocalizations. Severe enteritis, hepatic changes and generalized congestion in other organs of the body were seen upon post-mortem examination. A chronic lethal dose was calculated to be less than 0.63% of body weight. Shealy and Thomas (1928) presented a more complete description of the toxic effects of sesbania in the domestic chicken. These included the observation of ruffled feathers, staggered gait and drooped wings. Many whole seeds passed through the gastrointestinal tract without being digested and could be recovered intact. Recovered seeds were equally toxic when fed to a fresh group of fowl. The comb of treated individuals was discolored and flaccid. Profuse diarrhea was a consistent finding and muscle twitching in different parts of the body was also noted. Dramatic weight loss in affected individuals was paralleled by extreme weakness. Post mortem examination showed severe inflammation of the proventriculus and ulceration of the lining of the gizzard. This ulceration led to a separation of the lining from its muscular layer. The intestinal tract was found to be inflamed and congested (Shealy and Thomas, 1928). Chickens have been shown to be good models for studying sesbania-induced toxicosis. They are sensitive to the toxins and large numbers of birds are readily available at defined ages for experimentation (Flory and Hébert, 1984).

The closely related *Sesbania punicea* was found to cause similar signs in chickens (Terblanche et al, 1966). In this case, hyperemia and hemorrhage of the proventriculus were characteristic of intoxication. Petechial hemorrhages were evident in the gizzard.
Congestion and cloudy swelling were seen in the kidneys and liver along with marked congestion of the lungs.

Pigeons, turkeys and ducks were similarly affected by *Sesbania punicea* (Terblanche *et al.*, 1966). Pigeons vomited and showed hyperemia of the proximal portion of the small intestine. Upon histological examination, advanced cloudy swelling in the kidneys and liver was observed in treated pigeons.

In rats, an acute lethal dose of *Sesbania punicea* was calculated to be 3.0 g / kg (i.e. producing death within 24 hours). The eyelids became inflamed with hyperemia and exudation from the conjunctiva. Necropsy findings were similar to those for other species, including mild to moderate congestion, pulmonary emphysema and hyperemia of the gut. Only posterior paralysis was observed in acute intoxication. Subacute signs included listlessness, anorexia and diarrhea. In many cases, lesions of the gastrointestinal tract were not present (Terblanche *et al.*, 1966).

The rabbit was shown to be sensitive to fresh flowers, dried flowers and the seeds of *Sesbania punicea*. Chronic exposure produced marked hyperemia around the edges of the eyelids 2 to 9 days post treatment (Terblanche *et al.*, 1966).

In an experiment using only a single horse, Terblanche *et al* (1966) showed a slight drop in hemoglobin concentration without a concomitant decrease in the hematocrit number. There was an increase in the amount of unconjugated bilirubin as well as a slight rise in the BUN. No changes in serum glutamate oxaloacetate transaminase, serum pyruvate oxaloacetate transaminase, serum alkaline phosphatase or total plasma protein were observed. The
liver was swollen and spotted with petechial hemorrhages along the coronary vessels and the spleen was markedly congested. This is in marked contrast to the results reported by Flory and Hébert (1984) in chickens. The latter had observed a severe drop in the concentration of serum proteins.

Pigs dosed with *Sesbania punicea* showed signs of anorexia and gastric hemorrhages. Dogs were less susceptible than other animal species to intoxication since ingestion was followed by vomiting (Terblanche et al., 1966). Marsh and Clawson (1920) had previously shown that guinea pigs were susceptible to the actions of *Sesbania drummondii*. At least one human fatality occurred in a small boy who drank an infusion made from the seeds of *Sesbania drummondii* (Ellis, 1975).

Studies on isolated chick intestinal and lung parenchymal tissue have been carried out to further elucidate the mechanisms of action of the toxic principles of *Sesbania drummondii* on smooth muscle (Venugopalan et al., 1984). These authors suggested that part of the toxic action of the plant may be mediated via inhibition of smooth muscle function. This inhibition was found to be most pronounced in isolated lung parenchymal strips.

**Chemistry:**

Several compounds have been isolated from *Sesbania drummondii*, but at this time only one has been shown to be toxic (Powell et al., 1984). Robey (1925) attempted to identify the major toxin of *Sesbania drummondii*. Many of the symptoms of sesbania poisoning are similar to those observed in intoxications with
alkaloid-like compounds. Although Robey was unsuccessful in isolating alkaloids, or alkaloid-like compounds, he did detect the presence of a compound with the same empirical structure as a neutral saponin. He also had evidence for the presence of several acidic saponins. However, such compounds are not normally considered to be toxic. Saponins are extremely irritating substances known to produce hemolysis of red blood cells. Several signs of sesbania intoxication are consistent with the effects caused by a saponotoxin. These signs include severe gastric enteritis (all species), vomiting (in dogs), anorexia, abdominal pain and diarrhea (Terblanche et al., 1966). Emmel (1944) suggested that the absorption of a saponotoxin from prolonged retention of the seeds in the gastrointestinal tract might be responsible for the increased hemolysis observed. The isolation of a saponin from *Sesbania drummondii* is interesting in light of the observation that in the horse (Terblanche et al., 1966), rat and chicken (personal observation) there was hemolysis following sesbania treatment. However, other clinical findings of sesbania intoxication do not support the hypothesis that a saponin is the sole toxin. The decrease in serum proteins (Flory and Hébert, 1984), changes in smooth muscle function (Venugopalan et al., 1984) and neurological signs (Shealy and Thomas, 1928) cannot be explained by the presence of a saponin as the major toxin in sesbania. The presence of a cyanogenic glucoside has been reported, but this has never been substantiated (Broughton and Hardy, 1939; Simpson and West, 1953).

Interest in this plant was again stimulated during the early 1980's as part of an investigation to isolate naturally occurring
antineoplastic agents of botanical origin (Powell et al., 1983). Subsequent research led to the isolation of three major compounds. Two of these are the alkaloid sesbanine and the alcohol drummondol (Figure 1). A third compound, sesbanimide, isolated in minute quantities was shown to have antineoplastic activity. The structure of this antineoplastic compound is shown in Figure 1. Sesbanimide has been reported to be similar in structure to the glutaride class of antibiotics, such as cycloheximide (Powell et al., 1984). Due to this similarity in structure and to the extremely small yield of this compound (2 g from 450 kg of seeds), the suggestion has been made that sesbanimide might be from a microbial source contaminating the seed material (Gorst-Allman et al., 1983). However, sesbanimide has been independently isolated from the closely related *Sesbania punicea* and its structure confirmed by Gorst-Allman et al. (1983) using NMR spectroscopy. Studies on the pathophysiology of sesbanimide in animals have not been published. It is unlikely that sesbanimide is the sole toxin of *Sesbania drummondii*. 
Figure 1: Structures according to Powell et al. 1979; Powell et al. 1984.
MATERIALS AND METHODS

Source of Toxic Material:
Seed pods were collected from various areas in Louisiana. Initially, the seeds were removed from the pods with forceps and the collected seeds ground in a 2 mm screen meshed Wiley mill. However, it was found that the ground seeds and pods had virtually the same toxicity as whole ground seeds. Therefore, ground seeds and pods were used for the remainder of the experiments except as indicated. The ground materials were kept in an air-tight amber glass container until needed.

Preparation of Extracts:
The whole extract was prepared by suspending ground seeds and pods (henceforth referred to simply as ground seeds) in 0.025M sodium bicarbonate, 0.2% sodium azide buffer (BBN buffer, pH 7.0) in a ratio of 1 part ground seeds to 5 parts of buffer. The seeds were soaked overnight at 4C and the extract recovered by vacuum filtration through 6 layers of cheesecloth. Concentration of the extract was reported as the volume of filtrate per gram of seeds. This was the crude extract was used for dosing of animals by gavage.

A 20K (46,000 x g supernatant) fraction was prepared in the following manner: ground seeds of Sesbania drummondii were suspended in BBN buffer in the ratio of 1 part seeds to 5 parts buffer and processed in a Waring blender for 9 minutes at the
highest speed with intermittent cooling of the vessel in an ice-water bath. The homogenate was centrifuged at 46,000 x g (20,000 r.p.m.) for 20 minutes at 4°C in a Beckman J21B preparative centrifuge using a JA-20 rotor. The supernatant fraction was decanted through 6 layers of cheesecloth and the volume recorded. The concentration of this extract was expressed as the ml of extract recovered per gram of seeds.

pH Stability and Fractionation:

A 20K fraction was adjusted to the desired pH (2, 4, 6, 8, 10 or 12) with either 11.6 N HCL or 30.0 N NaOH to minimize volume changes. Precipitate formed was removed by centrifugation at 46,000 x g for 20 minutes. Both supernatant and pellet fractions were recovered. The supernatants were adjusted to pH 7.0 and the precipitates were resuspended to their original starting volumes with BBN buffer (pH 7.0) prior to dosing.

Dialysis:

Whole extract or 20K fraction was dialyzed against 200 volumes of BBN buffer (pH 7.0) in dialysis tubing with a molecular weight cutoff of 20,000 (Union Carbide, Chicago, IL). All fractions were dialyzed at 4°C for 24 hours with three buffer changes.

Concanavalin-A Sepharose™ and Sephadex™ G-25:

Five grams of Sephadex™ G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden) were swollen overnight in BBN buffer (pH 7.0). A slurry was prepared by mixing 10 ml of the 20K fraction with
swollen Sephadex™ G-25. This slurry was stirred for 4 hours at room temperature, vacuum filtered through Whatman #1 filter paper and used directly to dose chickens. Concanavalin-A Sepharose™ (Sigma Chemical Co., St. Louis, MO) was prepared in an analogous manner.

**Agarose:**

Agarose (Sigma Chemical Co., St. Louis, MO) was swollen overnight in BBN buffer (10 gm / 100ml of BBN buffer, pH 7.0). The gel was recovered by vacuum filtration through Whatman #1 filter paper and gently mixed with 20K fraction in a 1:1 ratio with intermittent stirring at room temperature for four hours. This mixture was filtered through Whatman #1 filter paper. The filtrate was used to dose animals. An equivalent volume of alpha-methyl-gluco side (10mg / ml) in BBN buffer (pH 7.0) was added to the filtered agarose gel, permitted to mix for 4 hours at room temperature and filtered as described above. This filtrate was used to dose chickens.

**Temperature Stability:**

A 20K fraction was frozen overnight at -20C (slow freeze), thawed at room temperature and used for dosing studies. In other experiments, the 20K supernatant was shell-frozen (fast freeze) in a dry-ice / methanol bath (approximate freezing temperature -60C) and lyophilized under vacuum using a Virtis uni-trap model 10-100 lyophilizer (Virtis, Gardner, NY). The residue was resuspended in a
volume of distilled water equivalent to the original volume of the 20K supernatant.

The 20K fraction was held at 37C, 50C or 80C for one hour. These extracts were cooled and used directly for dosing.

**Ultrafiltration:**

Each 20K fraction was sequentially filtered through 0.45 μm and 0.20 μm filters (Millipore Corp, Bedford, MA) sequentially prior to fractionation with a hollow fiber ultrafiltrator. Samples were prefiltered to avoid clogging the hollow fiber membranes with particulate material. The initial fractionation used a hollow fiber apparatus (Amicon CH4, Danvers, MA) with a molecular sieve limit of 1000. The <1000 MW filtrate was further separated at 4C into <500 and >500 MW fractions using a nitrogen driven (379.2 kp) Amicon ultrafiltration cell fitted with a YC 0.05 membrane (Amicon Corp., Danvers, MA). The >1000 and <1000 MW fractions were brought back to their original starting volumes with BBN buffer prior to dosing. The effective molecular weight cutoff of the YC 0.05 filter was 350 MW. The final protocol for the partial purification is shown in Figure 2.

**Cycloheximide:**

Cycloheximide, a glutaric antibiotic (Sigma Chemical Co., St. Louis, MO) was used to dose randomly selected mixed-sex, adult Sprague-Dawley rats. Animals were dosed with either 0.18 or 0.54 mg / kg per day for three days. A control group and a group
Figure 2: Protocol for the preparation of the <500 MW, ethyl acetate extractable toxin from *Sesbania drummondii*. 
receiving 0.3% body weight of a 20K supernatant fraction of *Sesbania drummondii* for three days were included.

**Attempted Isolation of Sesbanimide:**

A <500 MW fraction was adjusted to pH 2 with 11.6 N HCl and the precipitate separated by centrifugation at 46,000 x g for 20 minutes. The supernatant fraction was recovered and adjusted to pH 10 with 30N NaOH. After 30 minutes, precipitate formed was removed by a second centrifugation step at 46,000 x g for 20 minutes. The supernatant fraction was decanted and extracted three times with equal volumes of ethyl acetate in a separatory funnel. The ethyl acetate layers were removed and air-dried over CaCl₂. The dried extract was adjusted to the original starting volume with BBN buffer (pH 7.0) and used for dosing.

In a separate experiment, the 20K fraction was adjusted to pH 10 and extracted directly into ethyl acetate without prior purification and the resulting extract treated as above. Three layers resulted from this fractionation: aqueous, ethyl acetate and intermediate ethyl acetate-water. The ethyl acetate-water emulsion, which could not be separated by the addition of 1M NaCl, was removed intact and centrifuged at 46,000 x g for 20 minutes. Centrifugation did not separate the emulsion. This emulsion was air dried over a bed of CaCl₂.

Since the ethyl acetate fraction was found to be lethal, further purification aimed at the isolated of sesbanimide was attempted from the <500 MW fraction. As described above, the pH 10 adjusted <500 MW fraction was extracted into ethyl acetate and
taken to dryness. The residue was suspended in an equivalent volume of hot methanol (60°C) with rapid stirring. Cold dichloromethane (4°C) was added to this methanol suspension. As no precipitate was formed, the entire organic phase was taken to dryness and the residue prepared for dosing with the addition of BBN buffer. A flow diagram of this procedure is outlined in Figure 3.

**Thin Layer Chromatography:**

Thin layer chromatography was developed with a mobile phase of chloroform, methanol, and acetic acid (60:35:5) on fluorescence-suppressed silica gel plates (Whatman UV 256, Springhill, England). Samples of <500 MW and pure sesbanimide (a gift from Dr. Richard Powell, USDA Labs, Peoria IL) were spotted onto the plates in 5 μl aliquots using a 10 μl gas-tight syringe (Allometrics, Baton Rouge, LA). The developed TLC plates were visualized using the following colorimetric reagents.

Dragendorff solution: Solution A consisted of 2 gm of bismuth subnitrate in 25 ml glacial acetic acid diluted to 100 ml with distilled deionized water. Solution B consisted of 40 gm of potassium iodide made up to 100 ml with distilled water. Dragendorff's reagent consisted of 10 ml of solution A and 10 ml of solution B plus 20 ml of glacial acetic acid q.s. to 100 ml with distilled water. The final solution was orange-colored.

Mandelin reagent: 0.5 gm of ammonium vanadate was dissolved in 100 ml of concentrated sulfuric acid. Slight warming was necessary to completely dissolve the salt.
Figure 3: Isolation scheme for sesbanimide from partially purified extracts of *Sesbania drummondii*. 
Iodo-platinate reagent: Iodo-platinate (Whatman Chemical Co, Springhill, England) was prepared as a 0.15% potassium chloroplatinate, 3% potassium iodide spray reagent.

Potassium permanganate: 40 gm of potassium permanganate was dissolved in methanol and made up to 2000 ml with distilled water.

Concentrated sulfuric acid: 36 N was sprayed onto a plate and gently charred over a bunsen burner to visualize the chromatographed compounds.

UV: A hand held long and short wave variable ultraviolet light was used to detect fluorescent compounds in the mixture.

Animals:

Domestic White Leghorn chicks were hatched at the Department of Veterinary Science of Louisiana State University. Day old chicks were kept in heated brooders and fed Purina chick "Startena" and water ad libitum. No change in diet was effected throughout the experimentation. Heat was no longer necessary in the brooders after one week of age. Rhode Island Red chickens were occasionally used for experimentation. No breed differences were observed. Chickens were usually 2.5 to 3 weeks of age at the start of the experiment. Young chick groups (immature) were chosen to be less than 14 days old, while 6-9 week old chickens were placed in mature chicken groups. Six day old chicks were used for studies with pure sesbanimide. For each experiment, all chickens were of the same age (hatched on the same day) and were randomly divided into experimental groups.
All rats used in these studies were Sprague-Dawley males (except for BNF developmental studies which were prepubertal females) and were fed Purina rat chow and water ad libitum. Young rats were weaned at 19 days and used for experimentation from days 22 to 33 (prepubertal rats). Rats were randomly assigned to treatment groups. Adult male rats were used in the majority of experiments and were handled in the same manner as were young animals.

Weight calculations were reported as the percentage of the initial weight on any given day as described below.

\[
\left[ \frac{(W_x - W_i)}{W_i} \right] \times 100
\]

where \( W_x \) referred to the weight of the animals on any given day (\( X \)) and \( W_i \) was the initial weight of that animal at the start of the experiment.

**Determination of Serum Parameters:**

Serum bilirubin concentrations were quantitated using a commercial kit (Sigma Chemical Co., St. Louis, MO).

Immunodiffusion experiments were carried out as described by Fahey and McKelvey (1965). The agar buffer consisted of 300 mM
potassium phosphate buffer (pH 8.0) containing 1 M NaCl and 5.95 mM EDTA (disodium ethylene diamine tetraacetate); 15 gm of Noble agar (Difco, Detroit, MI) was added to 1 liter of buffer and heated until dissolved. The solution was cooled to 70°C and merthiolate (1:1000 w/v) was added as a preservative. The agar preparation was stored at 4°C in 10 ml aliquots for future use. Prior to use, the agar was melted at 95°C in a water bath. Antiserum (a dessicated sample of 2 ml of antiserum) was dissolved in 5 ml of agar buffer and warmed for 2 minutes at 56°C. The antisera (5ml) and heated agar (5ml) were quickly mixed and poured onto a calibrated immunodiffusion plate. The mixture was permitted to harden overnight at room temperature. Wells (3mm) were cut into the agar using a commercial template and filled with either standard serum samples for calibration or with test samples. Plates were incubated in a humidified chamber at room temperature for 24 hours and antibody-antigen precipitating diameters recorded. A standard curve was plotted on semi-log paper as the concentration of protein versus ring diameter after 24 hours.

Serum electrophoresis (Davis, 1964) was carried out using standardized cellulose acetate backed plates (Helena Laboratories, Beaumont, TX). Plates were electrophoresed for 20 minutes at 100 volts. Proteins were stained according to kit instructions with Ponceau S dye for 10 minutes and destained in 5% acetic acid. Protein intensity was quantitated with a transmittance densitometer following plate clearing in 30% ethyl acetate and 70% glacial acetic acid (Helena Laboratories, Beaumont, TX).
Liver Fractionation:

Livers were removed from experimental animals immediately following decapitation, rinsed once in ice cold 50 mM Tris, 1.15% KCl (pH 7.4) and patted dry for weighing. Following weighing, the livers were placed in ice-cold buffer, minced with scissors, rinsed once with fresh buffer and resuspended in fresh ice-cold buffer (pH 7.4) in a volume equivalent to twice the weight of the livers. They were homogenized using a SDT-1810 Tissuemizer (Tekmar, Cincinnati, OH) at a speed setting of 1/2 for 30 seconds. The homogenate was centrifuged at 7,800 × g for 20 minutes to remove red blood cells, cellular debris and mitochondria. The supernatants were decanted and recentrifuged at 102,000 × g for one hour in a Dupont-Sorval ultracentrifuge Model OTD-65 equipped with a Ti 865 fixed angle rotor. All the preceding steps were done at 4C. The cytosolic supernatant was decanted and its volume recorded. The microsomal pellet was carefully resuspended in ice-cold 1.15% KCL containing 10 mM EDTA. Microsomes were recovered after centrifugation at 102,000 × g for one hour. The microsomes were resusupended in the ice-cold KCl-EDTA buffer in a volume equivalent to the volume of the original cytosolic fraction. These microsomal fractions were used immediately for the spectral quantitation of cytochrome P-450 according to the method of Guengerich (1982).

Sulfhydryl Group Availability:

Sulfhydryl group availability (SH) was quantitated in the following manner. A standard curve was constructed using various glutathione concentrations. Each cuvette contained 1.45 ml of 0.5
mM dithiobisnitrobenzoic acid, 1.50 ml of distilled water and 50 µl of a known concentration of glutathione. The reaction was allowed to proceed for 5 minutes after which the absorbance was measured at 405 nm. The absorbance versus glutathione concentration was then plotted as a standard curve. Samples were reacted in a similar manner and their sulphydryl availability determined from the glutathione standard curve.

**Enzyme Activity Assays:**

Lactic dehydrogenase (LDH) was assayed by the method of Reeves and Fimognari (1966) with the following modifications. The buffer used in the assay was 0.05 M potassium phosphate (pH 7.4) containing 1% BSA and 0.15 M NaCl. Substrates were sodium pyruvate (0.226 M) and NADH (7.5 mM). A general stock solution consisted of the following: 3.0 ml of a 1% solution of BSA, 3.0 ml of phosphate buffer, 0.2 ml of pyruvate solution, 1.42 ml of NADH solution and 15.3 ml of distilled water. To each 3.0 ml cuvette, 1.45 ml of general stock solution and 1.35 ml of distilled water were added. The reaction was initiated by the addition of 50 µl of the enzyme preparation. The total assay volume was 3.0 ml. The reaction was assayed at 340 nm against a reagent blank in a Bausch and Lomb 2000 spectrophotometer at room temperature. The sources of enzyme were serum, liver cytosol or liver microsomes. Enzyme activity was calculated from the disappearance of NADH using an extinction coefficient of 6.22 x 10^6 liters / mole-cm.
Pyruvate kinase was assayed according to the method of Flory and Koepppe (1973). A 50 mM potassium phosphate buffer was adjusted to pH 7.5 by the addition of 1M NaOH and the concentration of potassium in this buffer was made to 90 mM with the addition of 40 mM KCl. The buffer also contained 1 mM 2-mercaptoethanol. The reaction mixture consisted of 0.145 mM NADH, 5 mM MgSO$_4$, 0.6 mM phosphoenolpyruvate (PEP), 1.5 mM adenosine diphosphate (ADP) and lactate dehydrogenase (final concentration, 50 units / ml) from rabbit muscle (Sigma Chemical Co., St. Louis, MO). Total volume of the assay was 3.0 ml and the reaction was initiated by the addition of 50 µl of test serum. The reaction cuvette was assayed against a reagent blank containing everything but serum. The samples were assayed in the presence and absence of fructose 1,6 (bis) phosphate (FBP, 1 mM). A total of 10 µl of the FBP was added to the assay cuvette. All reactions were carried out at room temperature for one minute and assayed at 340 nm. Enzymatic activity was measured as the disappearance of NADH using an extinction coefficient of $6.22 \times 10^6$ liters / mole-cm.

Glucose 6-phosphate dehydrogenase (GPDH) was assayed according to the method of Langdon (1966). The reagents used were: 1 M Tris-chloride buffer (pH 7.5), 0.025 M glucose 6-phosphate, 0.002 M NADPH and 0.2 M MgCl$_2$. A 0.1 ml aliquot of each reagent was added to 2.55 ml of distilled water and 50 µl of enzyme added to initiate the reaction. The final volume of the assay mixture was 3 ml. The reaction rate was monitored at 340 nm for 1 minute in a Bausch and Lomb 2000 spectrophotometer at room temperature. Units of enzyme activity were defined as the quantity of enzyme
that catalyzed the reduction of 1 μmole of NADP / minute using an extinction coefficient of 6.22 \times 10^6 \text{ liters / mole-cm}.

Glutathione peroxidase was quantitated by the method of Wendel (1981). Assay cuvettes contained 100 μl of potassium phosphate buffer (0.25 M, pH 7.0) containing 2.5 mM Na₂EDTA, 2.5 mM NaN₃, 100 μl yeast glutathione reductase (6 μmoles / ml, Sigma Chemical Co., St. Louis, MO), 100 μl of reduced glutathione (10 mM), 100 μl of NADPH (2.5 mM) and 100 μl of enzyme solution diluted to a protein concentration of 0.3 mg / ml. The volume was adjusted to 2.9 ml with distilled water. This reaction mixture was preincubated at 37°C for 10 minutes. The reaction was initiated by the addition of 100 μl of cumene hydroperoxide. The final volume of the reaction mixture was 3.0 ml. The linear increase in NADP was recorded at 366 nm. The spontaneous rate of NADPH oxidation was assessed in the absence of the enzyme and subtracted from reaction rates. One unit of activity was defined as a change in absorbance of 0.868 / minute / mole of reduced glutathione (GSH). The concentration of GSH was taken as unity. This coupled assay did not permit the use of alternative substrates but was suitable for the determination of glutathione peroxidase activity in crude homogenates (Wendel, 1981).

The glutathione -S- transferase isozymes (GSH-T) were determined by the method of Habig et al (1974). Each cuvette contained 2 ml of potassium phosphate buffer (0.1 M, pH 6.5) with 1 mM GSH, 40 μl of the substrate (1-chloro,2,4-dinitrobenzene dissolved in ethanol) and 30 μl of enzyme preparation (1/100 dilution of liver
fractions with 0.1 M potassium phosphate buffer, pH 6.5 of liver fractions). The reaction was monitored at 340 nm for 3 minutes. The enzymatic activity was calculated using the extinction coefficient of 9.6 liters / mole-cm for the formation of the glutathione conjugate. The following modifications were made for the assay of alternate substrates. The conditions of the assay for using 1,2 dichloro,4-nitrobenzene were 1 mM substrate, 5 mM GSH and 0.1 M potassium phosphate buffer (pH 7.5). The enzymatic activity was quantitated at 345 nm using an extinction coefficient of 8.5 liter / mole-cm for the glutathione adjunct. The assay of 4-trans-phenyl, 3-buten-2-dione which demonstrates activity for alpha, beta unsaturated ketones as substrates, was performed in the following manner (Ukagi et al, 1985). The assay cuvette contained 100 μl each of 0.1 M potassium phosphate buffer (pH 6.5), 0.05 mM 4-trans-phenyl, 3-buten-2-dione and 0.25 mM GSH. The reaction was initiated by the addition of 20 μl of a 1/100 dilution of microsome or cytosol with potassium phosphate buffer. The final volume of the reaction was q.s. to 3.0 ml with distilled water. The reaction rate was monitored at 290 nm for one minute and enzymatic activity was calculated using the extinction coefficient of -24.8 liter / mole-cm for substrate loss. The thiolipase activity of glutathione-S-transferase was measured by the method of Lankin et al (1984). Each cuvette contained 1 ml of 0.3 M potassium phosphate buffer (pH 7.0), 50 μl of 0.03 mM GSH, 50 μl of 12 mM nitrophenyl acetate (substrate), 50 μl of 60 mM EDTA and 1 ml of distilled water. The reaction was initiated by the addition of either 50 μl of microsomal or cytosolic preparations. The reaction was
monitored at 405 nm for 1 minute. The extinction coefficient for
the formation of nitrophenol was $13.6 \times 10^6$ liters / mole-cm. A
summary of these assay conditions is found in Table 2.

Acetylcholinesterase (AChE) and pseudocholinesterase (PChE)
were assayed according to the method of Mount and Oehme (1981).
Each cuvette contained 1.45 ml of 0.5 mM dithiobisnitrobenzoic acid
in 0.1 M Tris-HCl (pH 7.4), 1.35 ml of distilled water and 100 µl of
sample. The reaction was initiated by the addition of 100 µl of the
substrate, butyrylthiocholine iodide (0.156 M). The reaction was
monitored spectrophotometrically at 405 nm at room temperature
for 1 minute. Enzymatic activity was calculated for the appearance
of the mixed disulfide using the extinction coefficient of $45.7 \times 10^{-6}$
liters / mole-cm. Total cholinesterase activity was assayed in an
analogous manner, using acetylthiocholine iodide (0.156 M) as
substrate. AChE activities were determined as the difference
between acetyl and butyrylthiocholine activities.

Alkaline phosphatase (APase) was assayed according to the
method of Bowers and McComb (1966). The assay cuvette contained
0.8 ml of 1.0 M Tris-HCl buffer (pH 10) and 0.1 ml of 0.01 M para-
nitrophenylphosphate. The reaction was initiated by the addition of
100 µl of enzyme preparation and monitored at room temperature,
spectrophotometrically at 405 nm. Total volume of the reaction
mixture was 1.0 ml. An extinction coefficient of $13.6 \times 10^6$ liters /
mole-cm was used to calculate enzyme activity for the formation of
para-nitrophenol.

Gamma glutamyl transpeptidase (GGT) was assayed using a
commercial kit (Sigma Chemical CO., St Louis, MO). This assay
<table>
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<th>Substrate</th>
<th>Conc. (mM)</th>
<th>GSH (mM)</th>
<th>pH</th>
<th>lambda (nm)</th>
<th>Extinction Coefficient liters / mole-cm</th>
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<td>DCNB</td>
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<tr>
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DCNB = 1,2-dichloronitrobenzene  
DNCB = 1-chloro,2,4-dinitrobenzene  
PBD = 4-trans-phenyl, 3 buten-2-dione  
NPA = para-nitrophenylacetate

quantitates the liberation of para-nitroanisole from gamma glutamyl-para-nitroanisole. The para-nitroanisole is diazotized to form a pink azo dye, the concentration of which is proportional to the GGT activity.

**Protein Determinations:**

Serum proteins were routinely monitored by means of a temperature compensated hand-held refractometer (American Optical, Keene, NH). Liver cytosolic and microsomal protein concentrations were quantitated by the biuret method as described by Layne (1957). The biuret reagent was prepared by the addition of 6 gm of Na-K tartrate, 1.5 gm CuSO₄.5H₂O, 30 gm of NaOH and distilled water to a solution volume of 1 liter. Total assay volume was 5.0 ml consisting of 4.0 ml of assay reagent and 1.0 ml of protein sample. Bovine serum albumin (BSA) was used as a protein standard at the following concentrations 0, 1, 3, 6 and 10 mg/ml. Standard curves were constructed from duplicate samples and measured on a spectrophotometer at 500nm. Unknown protein concentrations were derived from the BSA standard curve.

**Histological Preparation:**

Livers were removed from rats immediately following decapitation and cut to an appropriate size with a fresh razor blade. The tissue was washed twice with 0.85% saline and placed in 10% formalin overnight.
Specimens were trimmed to fit a 5 mm x 10 mm x 2 mm molding cup. Samples were dehydrated twice in each of the following concentrations of ethanol: 70, 80, 90 and 95%. Samples were maintained in each dehydrating stage for 10 minutes. Total dehydration time was one hour.

Infiltration medium consisted of 5 parts 2-butoxy ethanol in 50 parts of hydroxy ethyl methacrylate butoxy-ethanol. Samples were polymerized at room temperature for two days. The specimens were removed from their respective molds and sectioned on a JB 4 ultramicrotome (Sorval, Dupont CO., Wilmington, DE) at 3 microns of thickness. Sections were dried overnight at 60C and cooled for 15 minutes prior to staining.

Sections were stained in Gill's hematoxylin III preparation (Polysciences, Warrington, PA) for 45 minutes and rinsed in tap water (20 dips each specimen). Nucleus staining was intensified by placing each specimen in Scott's tap water substitute (0.2 gm NaHCO3, 1 gm MgSO4.7H2O made up to 100 ml with water) for 25 minutes. Specimens were rinsed 20 times in tap water and counterstained with 1% eosin Y in 70% ethanol (Polysciences, Warrington, PA) for 25 minutes. The samples were dehydrated in absolute ethanol (10 dips / sample) and cleared in xylene. Cover slips were mounted over each specimen with Pro-Tex mounting media (Lerner Laboratories, New Haven CT).

**Transmission Electron Microscopy:**

Livers were immediately removed from decapitated rats and sliced into 1 mm blocks. Samples were fixed in 1.25%
glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at room temperature for one hour. The specimens were washed with 0.1 M sodium cacodylate buffer (pH 7.4) containing 5% sucrose for 10 minutes at room temperature. The washing procedure was repeated three times.

The specimens were stained with 2% uranyl acetate in 0.2 M sodium acetate buffer (pH 3.5) at room temperature for 2 hours and washed with deionized water 3 times for 10 minutes each.

The dehydration procedure was as follows: 30% ethanol (5 minutes), 50% ethanol (5 minutes), 70% ethanol (5 minutes x 2); 80% ethanol (10 minutes x 2), 90% ethanol (10 minutes x 2) and 100% ethanol (10 minutes x 3).

Specimens were infiltrated in the following manner: epoxy (Epon 812, Electron Microscopy Sciences, Fort Washington, PA) and propylene oxide in a ratio of 1:3 for 30 minutes, followed by epoxy and propylene oxide in a ratio of 2:2 for 1 hour. The epoxy resin concentrations were increased to a 3:1 ratio and samples maintained in this mixture for 30 minutes. This was followed by two changes of Epon 812 alone for 30 minutes each. The final polymerization step involved the addition of 15 µl of DMP-30 (polymerization catalyst) to Epon 812 for 30 minutes and post-staining the samples with 1% lead acetate.

Polymerization proceeded at 60C for 24 hours, after which the specimens were removed from their molds and the blocks trimmed and sectioned on a LKB 4 ultramicrotome (LKB Instruments, Gaithersburg, MD). Sections were placed on 200 mesh copper grids.
and photographs taken with a Zeiss EM 10 transmission electron microscope.

**Statistical Calculations:**

Individual results used for statistical analyses were the means of duplicate samples. Comparisons were made directly between control animals and *Sesbania drummondii* treated animals using the Student's T test. When the effects of more than two treatments were analyzed, an analysis of variance (ANOVA) was done. Since the age and sex of the animals were controlled, the analysis was of a one-way ANOVA. Pooled samples were treated as described by Steel and Torrie (1980). Comparisons involving more than 2 treatments were made using Tukey's $w$ procedure following analysis of variance. This test is applicable to pairwise comparison of means and makes use of the Studentized range.
RESULTS

Preliminary Studies on Toxin Isolation:

Extract Solubilization:

A comparison of weight loss in 2-3 week old chickens of mixed sex and mature rats of mixed sex dosed with whole extract and a 20K supernatant is shown in Figure 4. Birds dosed by gavage with whole extract or 20K supernatant exhibited equivalent weight loss (Figure 4). However, differences were noted in the mortality rate. The whole extract was more toxic (100% mortality within 4 days) than was the 20K supernatant, where 7 days of dosing were required for 100% mortality (Figure 4). The majority of the toxicity was found in the aqueous soluble fraction. No mortality was associated with the pellet fraction (Figure 4) although animals receiving sesbania pellet fractions failed to gain weight as rapidly as did control animals (Figure 4).

Rats showed similar weight losses upon dosing with either whole extract or 20K supernatant during the first two days of dosing. By days 3 and 4, however, rats dosed with the 20K supernatant had lost significantly less weight ($P<0.05$) than rats receiving the whole extract.

Serum protein and PChE (Pseudocholinesterase) activity in 10 day old chicks, quantitated after either 4 days of whole extract or 20K supernatant treatment, are shown in Table 3. Serum protein concentrations were depressed by both whole extract and 20K
Figure 4: Change in weight with day of dosing in chickens and rats dosed at 0.50% and 0.31% respectively with whole extract, 20K supernatant fractions or 20K pellet fractions (chickens) of *Sesbania drummondii*. 
TABLE 3

Circulating serum proteins and serum PChE in 10 day old chicks dosed with Sesbania drummondii extracts.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Protein (mg/ml)</th>
<th>ΔWeight (%)</th>
<th>PChE (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.65</td>
<td>35.60</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td>+0.02</td>
<td>+0.67</td>
<td>+-0.002</td>
</tr>
<tr>
<td>Whole Extract</td>
<td>2.72*</td>
<td>-25.50</td>
<td>0.022*</td>
</tr>
<tr>
<td></td>
<td>+0.05</td>
<td>+0.33</td>
<td>+-0.000</td>
</tr>
<tr>
<td>20K Supernatant</td>
<td>2.31*~</td>
<td>-36.70*~</td>
<td>0.007*~</td>
</tr>
<tr>
<td></td>
<td>+0.02</td>
<td>+0.78</td>
<td>+-0.000</td>
</tr>
</tbody>
</table>

Values are the means +- the SEM for n=10 animals

Control animals were untreated. Treated chickens were dosed for four days at 0.5% of their body weight/day.

Δ Weight is the percentage change in body weight at day 4 from the initial predose weight.

PChE (Pseudocholinesterase) units are expressed as nanomoles of substrate converted per minute per mg of protein at 22C.

Whole extract refers to ground seeds of Sesbania drummondii suspended overnight in BBN buffer (pH 7.0) in a ratio of 1 part seeds to 5 parts buffer and subsequently filtered through cheesecloth under vacuum. The resultant filtrate was used for dosing.

20K supernatant refers to the 46,000 x g (20,000 rpm) supernatant fraction of homogenized ground sesbania seeds.

* = means are significantly different from values obtained for control animals (P<0.05).

~ = means are significantly different from values obtained for whole extract treated animals.
supernatant treatments. The amount of decline was significantly (P<0.05) greater for those animals receiving the 20K supernatant. Similarly, weight loss was also found to be greater in 20K supernatant treated birds. The reverse was seen in older birds (Figure 4). Serum PChE activity was decreased by both treatments. Quantitatively, this decline was greatest for the 20K supernatant treated chickens.

Fasting:

Three week old chickens fasted over the same time frame as animals that had received sesbania extracts lost a substantial amount of body weight (32% over the first four days compared with 37% for sesbania treated birds). However, fasted chickens retained normal serum protein concentrations (3.4 mg/ml compared with 3.6 mg/ml for control animals, Table 4). The PChE activity in fasted chickens was unchanged compared with control birds, whereas, sesbania treated chickens, in a parallel experiment showed only 19% of the PChE activity observed for control chickens (not shown). Neither sesbania treatment nor fasting altered serum glucose concentrations. Fasted chickens lacked the petechial hemorrhages or smooth muscle changes that were typical of sesbania poisoned chickens.

pH:

The effects of pH fractionation on the toxicity of the 20K supernatant is shown in Table 5 (rats) and Table 6 (chickens). In rats, weight loss (-15.1%) was highest in those animals receiving
TABLE 4
Comparison of fasting and sesbania treatments in chickens on weight loss, serum protein concentration and PChE activity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum Protein (mg/ml)</th>
<th>ΔWgt (%)</th>
<th>Serum Glucose (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.6</td>
<td>+26.4</td>
<td>243.0</td>
</tr>
<tr>
<td></td>
<td>+0.4</td>
<td>+ 9.8</td>
<td>+-6.3</td>
</tr>
<tr>
<td>Fasting</td>
<td>3.2</td>
<td>-32.0 *</td>
<td>237.0</td>
</tr>
<tr>
<td></td>
<td>+0.2</td>
<td>+ -11.4</td>
<td>+ -2.2</td>
</tr>
<tr>
<td>Sesbania Extract</td>
<td>2.3</td>
<td>-37.0 *</td>
<td>249.0</td>
</tr>
<tr>
<td></td>
<td>+0.6</td>
<td>+ 8.3</td>
<td>+ -7.9</td>
</tr>
</tbody>
</table>

Results are the means of values for n=6 chickens +- the SEM.

* = means are significantly different from values obtained for control values (P<0.05).

Chickens were fasted or treated with 1% sesbania extract for 4 days.
### TABLE 5

Change in weight and mortality in rats dosed with pH adjusted 20K extracts of *Sesbania drummondii*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample</th>
<th>( \Delta )Weight (%)*</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>untreated</td>
<td>+2.50( \pm )0.55</td>
<td>0</td>
</tr>
<tr>
<td>20K (pH 7.0)</td>
<td>supernatant</td>
<td>-8.25( \pm )0.11</td>
<td>100</td>
</tr>
<tr>
<td>pH 2</td>
<td>supernatant</td>
<td>-15.10( \pm )0.53</td>
<td>40</td>
</tr>
<tr>
<td>pH 2</td>
<td>pellet</td>
<td>-9.67( \pm )0.19</td>
<td>17</td>
</tr>
<tr>
<td>pH 4</td>
<td>supernatant</td>
<td>-13.63( \pm )0.23</td>
<td>100*</td>
</tr>
<tr>
<td>pH 4</td>
<td>pellet</td>
<td>+2.35( \pm )0.12</td>
<td>20</td>
</tr>
<tr>
<td>pH 6</td>
<td>supernatant</td>
<td>+2.99( \pm )0.09</td>
<td>100*</td>
</tr>
<tr>
<td>pH 6</td>
<td>pellet</td>
<td>-10.67( \pm )0.14</td>
<td>80</td>
</tr>
<tr>
<td>pH 8</td>
<td>supernatant</td>
<td>+2.22( \pm )0.09</td>
<td>100*</td>
</tr>
<tr>
<td>pH 8</td>
<td>pellet</td>
<td>-11.37( \pm )0.42</td>
<td>80*</td>
</tr>
<tr>
<td>pH 10</td>
<td>supernatant</td>
<td>-4.39( \pm )0.24</td>
<td>80</td>
</tr>
<tr>
<td>pH 10</td>
<td>pellet</td>
<td>-0.04( \pm )0.13</td>
<td>40</td>
</tr>
<tr>
<td>pH 12</td>
<td>supernatant</td>
<td>+6.33( \pm )0.17</td>
<td>17</td>
</tr>
<tr>
<td>pH 12</td>
<td>pellet</td>
<td>+6.42( \pm )0.43</td>
<td>0</td>
</tr>
</tbody>
</table>

All rats were dosed at 0.3% of their body weight per day for 4 days unless otherwise noted. There were five animals per group.

* = means are significantly different from values obtained for control animals (untreated, P<0.05).

* = means are significantly different from values obtained for animals treated with unfraccionated 20K supernatant.

1 = % mortality after two days of dosing

2 = mortality after 1 day of dosing
TABLE 6
Change in weight and mortality rates in chickens dosed with pH adjusted 20K supernatant extracts of *Sesbania drummondii*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fraction</th>
<th>Δ Weight (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>+31.60 ± 0.38</td>
<td>0</td>
</tr>
<tr>
<td>20K (pH 7.0)</td>
<td>supernatant</td>
<td>-32.60* ± 0.11</td>
<td>100</td>
</tr>
<tr>
<td>pH 2</td>
<td>supernatant</td>
<td>-32.60*1 ± 0.57</td>
<td>20</td>
</tr>
<tr>
<td>pH 2</td>
<td>pellet</td>
<td>+15.60* ~ ± 0.13</td>
<td>0</td>
</tr>
<tr>
<td>pH 10</td>
<td>supernatant</td>
<td>-32.80*2 ± 0.19</td>
<td>60</td>
</tr>
<tr>
<td>pH 10</td>
<td>pellet</td>
<td>+19.10* ~ ± 0.10</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are the means ± SEM for n=10 animals per group.
1= values calculated on only 4 animals per group.
2= values calculated on only 2 animals per group.

Δ Weight indicates the % change in weight from the initial predose weight.

* = significantly different from control values.

~ = significantly different from values obtained for animals treated with 20K supernatant.
the pH 2 adjusted supernatant (Table 5), but this fraction produced only a 40% mortality compared with the untreated 20K supernatant (100% mortality). The pH 2 pellet was moderately toxic producing weight loss (~10%) and 17% mortality. The pH 4 pellet showed little or no toxicity, whereas its supernatant counterpart caused 100% mortality within 48 hours of the initial dose with a substantial weight loss (-13.6%). Despite the high mortality rate of the pH 6 supernatant (100% within 48 hours), weight loss was not observed in animals receiving this fraction (~+3.0%). The pH 6 pellet produced both a marked decline in weight (-10.6%) and a high mortality (80%). Like the pH 6 supernatant, the pH 8 supernatant produced no weight loss but caused 100% mortality within 48 hours. For fractions treated at more alkaline pH's, weight loss was minimal but still was higher for supernatants compared with pellet fractions. Mortality continued to be high for the pH 10 supernatant fraction but declined to only 17% for the pH 12 supernatant. Pellet fractions were generally less toxic than their supernatant counterparts.

The upper and lower pH ranges for the toxicity of sesbania 20K supernatants which had been used in rats (Table 5), were also examined in chickens. A similar pattern to what had been observed in sesbania treated rats was also seen in chickens, with the pellet fractions being far less toxic than the supernatants (Table 6; P<0.05). Once again, the unadjusted 20K supernatant produced 100% mortality within four days in chickens and was accompanied by severe weight loss (Table 6). Similar weight losses were observed for animals receiving either pH 2 supernatants or pH 10 supernatants. Again, the mortality was low with the pH 2
supernatant (20%) and high with the pH 10 supernatant (60%). The corresponding pellet fractions produced a slower rate of weight gain compared with control birds (Table 6) but there was no associated mortality (Table 6). As had been observed in the rats, most of the toxicity could be recovered in the supernatants. The greatest weight loss was observed with the pH 2 supernatant, although this difference was not significant (P>0.05) when compared with weight loss in 20K supernatant treated rats. Some species differences were noted, especially the substantial amount of weight loss incurred by chickens undergoing treatment with pH 10 supernatants. This fraction had not caused weight loss in the rat (Table 5).

**Dialysis:**

The administration of dialyzed extracts of *Sesbania drummondii* to mature chickens significantly increased (P<0.05) the weight loss compared with those birds receiving non-dialyzed extracts (Figure 5). After 3 days of dosing, there was an 8% difference in weight loss (22% for dialyzed versus 14% for those animals receiving non-dialyzed extracts).

**Concanavalin-A and Sephadex™ G-25:**

The effects of Concanavalin-A (Con-A, a lectin) and Sephadex™ G-25 (G-25, a compound known to be bound by certain lectins) on the toxicity of sesbania extracts in chickens were determined. Toxicity, based on weight loss, serum protein concentration and PChE activity, was reduced after either Con-A or G-25 treatment (Table 7). Following treatment, the 20K supernatant
Figure 5: The effect of dialysis on the toxicity of whole sesbania extracts.
TABLE 7

Protein, PChE and weight changes in chickens treated with 20K supernatant fractions and 20K fractions that had been treated with either Sephadex™ G-25 or Concanavalin A Sepharose.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein (mg/ml)</th>
<th>PChE (units)</th>
<th>Δ Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.40</td>
<td>0.081</td>
<td>+27.10</td>
</tr>
<tr>
<td></td>
<td>+0.08</td>
<td>+0.000</td>
<td>+1.44</td>
</tr>
<tr>
<td>20K</td>
<td>2.00*</td>
<td>0.029*</td>
<td>-18.30*</td>
</tr>
<tr>
<td></td>
<td>+0.10</td>
<td>+0.001</td>
<td>+1.18</td>
</tr>
<tr>
<td>Con A</td>
<td>3.00~</td>
<td>0.041*~</td>
<td>+9.30*~</td>
</tr>
<tr>
<td></td>
<td>+0.02</td>
<td>+0.001</td>
<td>+0.66</td>
</tr>
<tr>
<td>Seph.™ G-25</td>
<td>3.00~</td>
<td>0.044*~</td>
<td>+19.30*~</td>
</tr>
<tr>
<td></td>
<td>+0.01</td>
<td>+0.001</td>
<td>+1.28</td>
</tr>
</tbody>
</table>

Values are means ±SEM for n=6 animals per group.

Controls were untreated. Treated animals were dosed for 4 days at 1% of their body weight/day.

PChE units are expressed as nanomoles of substrate converted per minute per mg of enzyme.

Δ Weight is the percentage change in weight after 4 days from the initial predose weight.

Con A = Concanavalin A-Sepharose mixed as a slurry with the extract and stirred at room temperature for 2 hours, then filtered through Whatman#1 filter paper.

Seph.™ G-25 = Sephadex™ G-25 treated as a slurry in an analogous manner to the Con A experiment.

* = means are significantly different (P<0.05) from values obtained for control animals.

~ = means are significantly different (P<0.05) from values obtained for 20K treated animals.
no longer significantly lowered serum protein concentrations. PChE activity, after dosing with treated extracts, remained at about 1/2 control values regardless of the treatment. However, these values were significantly above those obtained for animals receiving the untreated 20K supernatant (P<0.05). Treatment of the 20K supernatant with Seph. G-25 reversed the weight loss although the average chicken weight remained at about 71% of control (untreated) animals. Con-A treatment also diminished the weight loss induced by 20K supernatant (34% of the control value).

**Temperature:**

The effects of freezing and freezing followed by dialysis on the toxicity of 20K supernatants were examined in rats (Table 8). Although weight loss was less in animals receiving frozen extracts (-18.3% versus -9.1% for 20K and frozen 20K supernatants, respectively), declines in serum protein concentration (4.64 and 4.00, respectively) and PChE activity (0.618 and 0.497, respectively) were larger than for those receiving unfrozen extracts of sesbania. Dialysis of a previously frozen extract was found to produce an extract which was much less toxic than the original frozen extract (Table 8). Similarly, lyophilization reduced both the degree of weight loss and the decline in PChE activity observed with the 20K supernatant (Table 8). In contrast, the lyophilized 20K supernatant produced a decrease in serum proteins greater than that achieved by the untreated 20K supernatant. Extracts of sesbania were shown to have no direct effect on the activity of purified horse serum PChE or
TABLE 8

The influence of freezing on the toxicity of the 20K supernatant fraction in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ΔWeight (%)</th>
<th>Protein (mg/ml)</th>
<th>PChE (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+18.17</td>
<td>5.86</td>
<td>0.836</td>
</tr>
<tr>
<td></td>
<td>+1.21</td>
<td>-0.04</td>
<td>+0.004</td>
</tr>
<tr>
<td>20K</td>
<td>-18.30*</td>
<td>4.64*</td>
<td>0.618*</td>
</tr>
<tr>
<td></td>
<td>+1.38</td>
<td>-0.03</td>
<td>+0.002</td>
</tr>
<tr>
<td>Frozen</td>
<td>-9.13*~</td>
<td>4.00*~</td>
<td>0.497*~</td>
</tr>
<tr>
<td></td>
<td>+0.17</td>
<td>-0.04</td>
<td>+0.003</td>
</tr>
<tr>
<td>Frozen/Dialyzed</td>
<td>-4.10*~</td>
<td>5.40~</td>
<td>0.513*~</td>
</tr>
<tr>
<td></td>
<td>+0.37</td>
<td>-0.03</td>
<td>+0.002</td>
</tr>
<tr>
<td>Lyophilized</td>
<td>-1.53*~</td>
<td>4.14*~</td>
<td>0.882~</td>
</tr>
<tr>
<td></td>
<td>+1.25</td>
<td>-0.05</td>
<td>+0.009</td>
</tr>
</tbody>
</table>

Values are means ± the SEM for n=8 animals per group.

Control animals were untreated. Rats were dosed at 0.5% of their body weights/day for three days.

PChE units are expressed as nanomoles of substrate converted per minute per mg of protein.

Δ Weight indicates the percentage change in weight at day 3 from the initial predose weight.

* = means are significantly different (P<0.05) from values obtained for control animals.

~ = means are significantly different (P<0.05) from values obtained for 20K treated animals.
The ability to induce weight loss was completely lost when 20K supernatants were heated for one hour at either 50 or 80°C (data not shown). Similarly, heating the extracts resulted in a loss of the ability to induce decreases in serum protein concentration in both chickens and rats. Values were obtained that were not significantly below values obtained for control (untreated) animals (Table 9). PChE activity remained below control values following heating but were significantly higher than the values obtained from animals that had received whole sesbania extract. In the rat, heated sesbania extracts caused a greater decline in serum protein concentrations than did untreated extracts (Table 9). As had been observed for the chicken, heated sesbania extracts continued to cause decline in PChE activity compared with control values but this decline was significantly less than that observed for the whole unheated extract (Table 9).

**Agarose:**

Treatment of 20K supernatants with agarose reduced the degree of weight loss determined after 2 days of treatment (Figure 6). The toxicity of the extract could not be restored by eluting the sesbania treated agarose with alpha-methyl-glucoside. In all cases, these treatments reduced overall weight loss compared with those chickens receiving the untreated 20K supernatant extracts (Figure 6).
TABLE 9

Serum proteins and PChE activity in chickens and rats dosed with heated extracts of *Sesbania drummondii*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CHICKEN</th>
<th>RAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum Protein (mg/ml)</td>
<td>PChE (units)</td>
</tr>
<tr>
<td>Control</td>
<td>3.28 ±0.05</td>
<td>1.088 ±0.005</td>
</tr>
<tr>
<td>Whole Extract</td>
<td>2.35* ±0.14</td>
<td>0.256* ±0.004</td>
</tr>
<tr>
<td>50C.</td>
<td>3.06 ±0.14</td>
<td>0.496*~ ±0.002</td>
</tr>
<tr>
<td>80C.</td>
<td>3.10 ±0.19</td>
<td>0.513*~ ±0.017</td>
</tr>
</tbody>
</table>

Values are the means ± the SEM for n=6 animals per group.

Whole extract = ground filtered seeds and pods of sesbania

50C or 80C = treatment temperature at which whole extract was subjected for 60 minutes.

Controls were untreated. Rats were dosed for 4 days at 0.5% and chickens dosed at 1.0% of their body weight/day.

* = means are significantly different from values obtained for control animals (P<0.05).

~ = means are significantly different from values obtained for animals receiving whole extracts (P<0.05).
Figure 6: The effects of agarose and alpha methyl-glucoside treatments on the toxicity of whole sesbania extracts in the chicken
Molecular Weight:

Molecular weight fractionation of 20K supernatants showed that the >1000 MW fraction failed to produce weight loss in treated chickens (Table 10), although animals receiving this extract did not gain weight as rapidly as did control chickens (+25.9% and +53.7%, respectively). Weight loss in chickens was observed when treated with a <1000 MW fraction (-41.5%, Table 10). The <1000 MW fractions demonstrated greater weight loss than did the <500 MW fraction (Table 10). Mortality was highest for those animals receiving the 20K supernatants (100%) followed by the <1000 MW (86%) and then the <500 MW fraction (67%, Table 10). The >1000 MW fraction produced zero mortality (Table 10). Compared with the control values significant decreases (P<0.05) in serum protein concentrations were achieved in those animals who had received the >1000 MW (2.64 mg protein/ml of serum [mg/ml]), the <500 MW (2.33 mg/ml) and the <1000 MW fractions (2.64 mg/ml). These values were significantly above (P<0.05) the values obtained for the 20K supernatant treated animals (2.00), but remained significantly below (P<0.05) values obtained for control (untreated) animals (3.75 mg/ml).

Serum PChE activity was unaltered in those animals receiving the >1000 MW fractions (Table 9). Those animals, which had been dosed with the <1000 MW fraction, showed significant declines in PChE activity (0.072, P<0.05). This value was proportionately less but still significant (P<0.05) for those animals receiving the <500 MW fraction (0.109, P<0.05, Table 10). The overall toxicity of the <500 MW fraction was less for the three
TABLE 10

Comparison of % weight, mortality, serum protein and pseudocholinesterase changes induced by ultrafiltration extracts of the 20K supernatant in chickens.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ΔWeight (%)</th>
<th>Mortality (%)</th>
<th>Protein (mg/ml)</th>
<th>PChE (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+53.7(^a)</td>
<td>0(^a)</td>
<td>3.75</td>
<td>0.164</td>
</tr>
<tr>
<td>20K</td>
<td>-34.2(^*)</td>
<td>100(^b)</td>
<td>2.00(^*)</td>
<td>0.091(^*)</td>
</tr>
<tr>
<td>&gt;1000 MW</td>
<td>+25.9(^*)</td>
<td>0(^a)</td>
<td>2.64(^*)</td>
<td>0.171(^*)</td>
</tr>
<tr>
<td>&lt;1000 MW</td>
<td>-41.5(^*)</td>
<td>86(^a)</td>
<td>2.00(^*)</td>
<td>0.072(^*)</td>
</tr>
<tr>
<td>&lt;500 MW</td>
<td>-19.9(^*)</td>
<td>67(^c)</td>
<td>2.33(^*)</td>
<td>0.109(^*)</td>
</tr>
</tbody>
</table>

Values are means ± the SEM for n = 10 animals per group.

Control animals were untreated. Ten day old chicks were dosed at 0.5% of body weight/day for six days. The chickens were dosed for three days with the 20K extract and for 4 days with the <500 MW fraction.

Molecular weight fractions were as follows: >1000 and < 1000 separated from the 20K extract by an Amicon hollow fiber device. The <500 MW fraction was separated from the <1000 fraction by the use of an Amicon YC 0.05 membrane.

Δ Weight is the % change change in the weight at day six from the initial predose weight.

% Mortality is calculated after six days of dosing except as follows: a= mortality at day 6 of dosing; b= mortality at day 3 of dosing; c = mortality at day 4 of dosing

PChE units are expressed as the nanomoles of substrate converted per minute per mg of protein.

\(^*\) = means are significantly different from control values (P<0.05).

\(^\sim\) = means are significantly different from 20K supernate values (P<0.05).
parameters examined (i.e. weight loss, serum protein concentration and PChE activity) compared with the <1000 MW fraction.

**Cycloheximide:**

The pathological features observed during cycloheximide poisoning or sesbania intoxication of treated rats are shown in Table 11A. The general pathology was similar for both compounds. However, important differences between the two treatments were noted especially in the histopathology (Table 11A). Serum protein concentrations were lowest for those animals receiving sesbania extracts (Table 11B). The decline in PChE activity for those animals receiving the sesbania extracts was greater than for those animals treated with either concentration of cycloheximide (Table 11B). PChE activity was decreased by both doses of cycloheximide tested but this decrease was not dose related. The activity of this enzyme was lower for sesbania treated animals than either cycloheximide or control animals (Table 11B).

Histological and electron microscopic examinations elucidated further differences between cycloheximide and sesbania treatments. No changes were observed in the histological appearance of liver removed from rats that had been treated with sesbania compared with control animals (Figure 7, 8). However, extreme vacuolization was observed in liver obtained from animals that had been dosed with 30% of the LD$_{50}$ of cycloheximide (Figure 9). No changes could be detected in sesbania treated cells in thin section (Figure 10, 11), with the possible exception of some slight vacuolization. Once again, those animals that had been treated
TABLE 11A

Comparison of the pathology induced by cycloheximide and crude extracts of *Sesbania drummondii* in rats - pathology.

<table>
<thead>
<tr>
<th>Control</th>
<th>Cycloheximide 10% LD$_{50}$</th>
<th>Cycloheximide 30% LD$_{50}$</th>
<th>Sesbania Whole Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>weight</td>
<td>moderate</td>
<td>greatest</td>
<td>moderate</td>
</tr>
<tr>
<td>gain</td>
<td>weight loss</td>
<td>weight loss</td>
<td></td>
</tr>
<tr>
<td>normal</td>
<td>gut hyperemia</td>
<td>gut hyperemia</td>
<td>gut hyperemia</td>
</tr>
<tr>
<td>normal</td>
<td>engorged stomach</td>
<td>engorged stomach</td>
<td>engorged stomach</td>
</tr>
<tr>
<td>Histo: liver</td>
<td>Histo: liver vacuolization</td>
<td>Histo: liver extreme vacuolization</td>
<td>Histo: liver normal</td>
</tr>
</tbody>
</table>

Observations on n=6 animals per group except for EM studies where only three animals were examined per group.

Cycloheximide in chickens gave identical results except that no electron microscopic or histological examinations were undertaken.

Protocol: Since sesbanimide isolated from *Sesbania drummondii* has been reported to share some structural similarities to glutaride-like antibiotics and pure sesbanimide was unavailable, rats were dosed with 10% (0.18 mg / kg per day) or 30% (0.54 mg / kg per day of the LD$_{50}$ of cycloheximide in rats and the pathology compared to a group of rats that had received whole sesbania extracts. All animals were dosed for three days.
### TABLE 11B

Comparison of the pathology induced by cycloheximide and crude extracts of *Sesbania drummondii* in rats - serum chemistry.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum Protein (mg/ml)</th>
<th>PChE Activity (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.62 ± 0.23</td>
<td>0.370 ± 0.200</td>
</tr>
<tr>
<td>10% LD&lt;sub&gt;50&lt;/sub&gt; cycloheximide</td>
<td>4.48~ ± 0.26</td>
<td>0.170*~ ± 0.015</td>
</tr>
<tr>
<td>30% LD&lt;sub&gt;50&lt;/sub&gt; cycloheximide</td>
<td>4.28~ ± 0.57</td>
<td>0.171*~ ± 0.004</td>
</tr>
<tr>
<td>Whole Extract</td>
<td>3.75* ± 0.31</td>
<td>0.148* ± 0.003</td>
</tr>
</tbody>
</table>

General serum chemistry for n=6 animals per group.

Cycloheximide in chickens gave identical results except that no electron microscopy or histological work was undertaken.

PChE units are expressed as nanomoles of substrate converted per minute per mg of protein.

* = means are significantly different (P<0.05) from values obtained for control animals.

~ = means are significantly different (P<0.05) from values obtained for animals receiving sesbania extracts.
Figure 7: Histological section of control rat liver stained with Hematoxylin A III and Eosin Y for 25 minutes each. 1800 x magnification

A - nucleus
B - sinusoidal space

Figure 8: Histological section of liver from sesbania treated rats stained with Hematoxylin III and Eosin Y for 25 minutes each. 1600 x magnification.

A - nucleus
B - sinusoidal space
Figure 9: Histological section of liver from cycloheximide (30% LD 50) treated rats stained with Hematoxylin III and Eosin Y for 25 minutes each. 1600 x magnification.
A - nuclues
B - lumen
C - vacuolization
Figure 10: Thin section transmission electron photomicrograph of control rat liver post-fixed in osmium tetroxide and stained with 1% uranyl acetate. Final magnification is 45,000.

A - mitochondrion  
B- dark staining inclusion body (possibly lipid)  
C- small vacuole

Figure 11: Thin section transmission electron photomicrograph of liver from sesbania treated rats post-fixed in osmium tetroxide and stained with 1% uranyl acetate. Final magnification is 22,500.

A - nucleus  
B - dark staining inclusion body (possibly lipid)  
C- Golgi ?  
D- small vacuole
with cycloheximide displayed a completely different appearance (Figure 12). TEM (transmission electron microscopy) confirmed the extreme vacuolization initially observed in histological sections. These distended vacuoles were filled with a flocculant precipitate.

**Sesbanimide:**

Sesbanimide was lethal to 6 day old chicks (average chick weight 50 to 70 gm) at a concentration of 0.25mg / ml. Each bird received 1 ml of this sesbanimide solution via gavage. Birds dosed at this concentration exhibited substantial weight loss (15%), decreased serum protein concentrations (42% of control) and decreased PChE activity (28% of control, data not shown). This was accompanied by generalized weakness and smooth muscle inhibition as evidenced by the full and distended appearance of the crops of treated birds. There was 100% mortality within 24 hours. Sesbanimide made up in BBN buffer and kept refrigerated continued to cause mortality in chickens for up to seven days even at a reduced dose of 1.04 mg / kg chick weight.

Sesbanimide treated birds displayed all of the clinical signs associated with sesbania toxicosis except that the onset of death was extremely rapid. Thin layer chromatography on silica plates, followed by charring with sulphuric acid, indicated that sesbanimide might be present in crude extracts of sesbania. Pure sesbanimide was found to be too labile for analysis by gas chromatography. HPLC monitoring gave the following results: pure sesbanimide gave two peaks, a minor peak at 7.4 minutes and a major peak at 9.56 minutes retention time. The height of the minor peak was increased
Figure 12: Thin section electron photomicrograph of liver from cycloheximide (30% LD 50) treated rats post-fixed is osmium tetraoxide and stained with 1% uranyl acetate. Final magnification is 20,000
A - mitochondria
B - moderately large vacuole
C - vacuole with flocculant precipitate
(possibly protein)
when sesbanimide was permitted to stand in aqueous solution at 4C for 3 days. HPLC analysis of a partially purified sesbania extract (Figure 2) contained eight separate peaks. The most abundant peak had a retention time of 7.35 minutes and was similar to the minor peak found in the purified sesbanimide preparation. On the basis of relative peak heights, the partially purified sesbania extract contained 22.3 times the concentration of the 7.4 minute peak found with the sesbanimide sample (minor sesbanimide peak). The only other peak from the partially purified sesbania extract comparable to the sesbanimide sample corresponded to a retention time of 9.79 minutes. This would represent about 1/3 the total concentration of the major peak in the sesbanimide sample. However, when comparisons were made for relative toxicity (estimated on the ability of either sesbanimide or the partially purified sesbania fraction to cause death), the <500MW fraction was found to be far less toxic (30% mortality) when compared with the sesbanimide preparation (100% mortality). Under these conditions, doses were calculated on the basis of equivalent HPLC peak heights.

Sesbanimide would not crystallize from the partially purified sesbania extract from a mixture of hot methanol and dichloromethane nor was the toxicity of the extract maintained under these conditions.
Studies on the Mechanisms of Sesbania Action(s):

Pathophysiology:

The common pathological findings in rats and chickens treated with extracts of *Sesbania drummondii* included depression and listlessness. The body fat of affected chickens and rats was almost entirely depleted. The eyes of treated individuals were commonly exudous and accompanied by some crustiness. All animals had cold extremities although their body temperatures remained normal over the 4 day dosing regimen (not shown). Postmortem examination showed a distended, bloated stomach. The lower gastrointestinal tract was hyperemic and empty with vasodilatation. These gastrointestinal changes were accompanied by diarrhea. The hematocrits of sesbania treated chickens and rats were frequently depressed. Other postmortem observations were an extremely friable, mottled liver and frequently the kidneys of treated rats were gun-metal gray with occasional mottling.

In chickens, the signs of terminal sesbania poisoning included behavioral deficits, especially an inability to stand or hold up their heads. The combs of male birds were frequently discolored, swollen and flaccid. In the terminal stages, birds displayed rapid shallow breathing. The feathers were ruffled and the birds died without struggle. There was generalized muscular wasting in chickens, particularly of the pectoral muscle which was often translucent. The leg muscles of sesbania treated chickens were wasted and dark in color with multiple petechial hemorrhages. The crops of birds treated with sesbania extracts were full and these birds invariably
regurgitated mucus when handled, particularly after the second day of dosing. The gall bladders of affected birds were turgid. At necropsy, the tissues of treated birds appeared dehydrated.

**Weight Loss:**

In chickens, weight loss in response to sesbania treatment was evident from the first day of dosing (Table 12). In rats, which received one half the dose administered to chickens (0.5% versus 1.0% of the body weight/day), significant weight loss did not occur (P>0.05) until day three of dosing. However, weight gain in sesbania treated rats was significantly below (P<0.05) that of controls by day two of dosing (Table 12).

In parallel with weight loss, the liver weights of sesbania treated rats and chickens were found to be lowered in comparison with control animals (Table 13). However, in spite of comparable decreases in both body weight and liver weight, the liver to body weight ratios remained relatively constant with treatment (Table 14). In the chicken, sesbania treatment elevated the cytosolic protein/liver weight fraction (Table 14) with only a slight non-significant (P>0.05) increase in the microsomal protein/liver weight ratio. Rats undergoing sesbania treatment showed a reversal in this pattern of protein ratios. The microsomal protein / liver ratio was found to be significantly higher (P<0.05) than the values obtained for control animals. The slight increase in the cytosolic / liver ratio from sesbania treated rat liver was not significant (P>0.05, Table 14).
TABLE 12
Comparison of weight change induced by crude extracts of *Sesbania drummondii* in chickens and rats.

<table>
<thead>
<tr>
<th></th>
<th><strong>CHICKEN</strong></th>
<th></th>
<th></th>
<th><strong>RAT</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ Weight (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>5.50</td>
<td>-3.12*</td>
<td>15.00</td>
<td>11.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+-0.20</td>
<td>+-0.23</td>
<td>+-0.40</td>
<td>+-0.64</td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>9.93</td>
<td>-4.97*</td>
<td>30.33</td>
<td>12.45*~</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+-0.24</td>
<td>+-0.29</td>
<td>+-0.06</td>
<td>+-0.18</td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>18.59</td>
<td>-10.65*</td>
<td>36.00</td>
<td>6.20*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+-0.43</td>
<td>+-0.29</td>
<td>+-0.56</td>
<td>+-0.10</td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>23.69</td>
<td>-16.89*</td>
<td>44.00</td>
<td>-8.00*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+-0.60</td>
<td>+-0.45</td>
<td>+-0.83</td>
<td>+-0.93</td>
<td></td>
</tr>
</tbody>
</table>

Values are the means ± the SEM for n = 22 chickens and n = 6 rats per group.

Controls were untreated for both species. Chickens were dosed at 1.0% of the body weight/day while rats received 0.5% of the body weight/day for four days.

Δ Weight indicates the % change in weight at each day from the initial predose weight. Toxicity is reported as the Δ% weight change from the initial weight.

* = means are significantly different from the values obtained for control animals (P<0.05).

~ = means are significantly different from the previous day 's weight change (P<0.05).
TABLE 13
Comparisons of liver weights in chickens and rats dosed with 20K supernatant fractions of *Sesbania drummondii*.

<table>
<thead>
<tr>
<th>Liver Weight (gms)</th>
<th>CHICKENS</th>
<th>RATS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td>8.74</td>
<td>6.64*</td>
</tr>
<tr>
<td></td>
<td>+-0.09</td>
<td>+-0.03</td>
</tr>
</tbody>
</table>

Values are the means ± the SEM for n= 22 chickens and n= 9 rats per group.

Controls were untreated for both species. Chickens were dosed at 1.0% body weight / day for three days while rats were dosed at 0.5% of body weight / day.

* = means are significantly different from values obtained for control animals (P<0.05).
TABLE 14

Comparison of liver protein to liver weight ratios in chickens and rats dosed with 20K superantant fractions of *Sesbania drummondii*.

<table>
<thead>
<tr>
<th>Liver Protein / Liver Weight Ratio (mg microsomal or cytosolic protein / gm liver weight)</th>
<th>Chickens</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Microsomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.56</td>
<td>2.78</td>
</tr>
<tr>
<td></td>
<td>±0.04</td>
<td>±0.04</td>
</tr>
<tr>
<td>Cytosol</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.51</td>
<td>6.84*</td>
</tr>
<tr>
<td></td>
<td>±0.00</td>
<td>±0.01</td>
</tr>
</tbody>
</table>

Values reported as the mean ± the SEM for n=12 chickens per group and n = 6 rats per group.

Control animals were untreated. Chickens were dosed at 1.0% of their body weight/day for three days while rats were dosed at 0.5% of their body weight/day.

* = means are significantly different from values obtained for control animals (P<0.05).
Serum sodium concentrations were unchanged in chickens, but were significantly lowered in sesbania treated rats ($P<0.05$, Table 15). In contrast, there was a significant decrease in potassium concentration in sesbania treated chickens ($P<0.05$, Table 15) which was not observed in sesbania treated rats. The blood pH of treated chicken was significantly increased (alkaline, $P<0.05$) but significantly depressed (acidic, $P<0.05$) in blood obtained from sesbania treated rats.

The blood from treated animals showed a greater tendency toward hemolysis than did blood obtained from untreated animals. The blood hematocrit was depressed in both treated chickens and rats. However, this decrease was only significant ($P<0.05$) for blood obtained from sesbania treated chickens (Table 15).

**Electrophoresis:**

Table 3 shows that serum protein concentrations fell in response to sesbania treatment in both chickens and rats. Serum from sesbania treated chickens showed decreased albumin (Table 16). The alpha 1 fraction fell slightly while the alpha 2 fraction rose slightly in response to sesbania treatment. The beta fraction rose somewhat in the chicken following sesbania treatment. The gamma globulin concentration fell. Changes were observed in the serum of sesbania treated rats (Table 17) due mainly to the depletion of the albumin fraction. Prealbumin represented a substantial fraction in the sesbania treated rats whereas it had been negligible in control rats (Table 17). The alpha1 fraction was
TABLE 15

Sesbania induced changes in serum and blood parameters in chickens and rats.

<table>
<thead>
<tr>
<th></th>
<th>CHICKENS</th>
<th>RATS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Na+ meq</td>
<td>146.00</td>
<td>145.62</td>
</tr>
<tr>
<td></td>
<td>±0.22</td>
<td>±0.24</td>
</tr>
<tr>
<td>K+ meq</td>
<td>4.61</td>
<td>3.05*</td>
</tr>
<tr>
<td></td>
<td>±0.10</td>
<td>±0.06</td>
</tr>
<tr>
<td>pH</td>
<td>7.355</td>
<td>7.598*</td>
</tr>
<tr>
<td></td>
<td>±0.007</td>
<td>±0.002</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>42.67</td>
<td>36.77*</td>
</tr>
<tr>
<td></td>
<td>±0.36</td>
<td>±0.68</td>
</tr>
</tbody>
</table>

Values are the means ± the SEM for n = 9 chickens per group. Rat values are for n = 30 animals per group except for pH values where n = 5 animals per group and hematocrit where n = 10 animals per group.

Control animals were untreated. Chickens were dosed at 1.0%, rats at 0.5% of their body weights/day, respectively for three days.

* = means are significantly different from the values obtained for control animals (P<0.05).
### TABLE 16

Electrophoretic profile of control chicken serum and serum derived from animals that had been treated with 20K supernatant sesbania extracts.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>TOTAL PROTEIN (%)</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>50.3</td>
<td>9.3</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>+/-2.7</td>
<td>+/-0.8</td>
<td>+/-1.3</td>
</tr>
<tr>
<td>Alpha 1</td>
<td>37.1*</td>
<td>7.7</td>
<td>16.8*</td>
</tr>
<tr>
<td></td>
<td>+/-3.2</td>
<td>+/-0.3</td>
<td>+/-0.9</td>
</tr>
</tbody>
</table>

Values expressed as % of the total protein for n=6 chickens per group. Initial protein concentrations are not identical for both control and treated chickens but are representative of the distribution of serum proteins between the two groups.

Controls were untreated. Chickens were dosed for three days at 1.0% of body weight/day for three days.

* = means are significantly different from control values (P<0.05).
TABLE 17

Electrophoretic partition of control rat serum and serum derived from rats treated with 20K supernatant extracts of *Sesbania drummondii*.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Control (%)</th>
<th>Treated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prealbumin</td>
<td>0.3 (+0.2)</td>
<td>12.5* (+4.6)</td>
</tr>
<tr>
<td>Albumin</td>
<td>56.7 (+2.2)</td>
<td>43.5* (+1.5)</td>
</tr>
<tr>
<td>Alpha 1</td>
<td>15.0 (+1.2)</td>
<td>9.9* (+0.6)</td>
</tr>
<tr>
<td>Alpha 2</td>
<td>4.8 (+1.1)</td>
<td>10.9* (+0.6)</td>
</tr>
<tr>
<td>Beta 1</td>
<td>13.1 (+2.0)</td>
<td>9.6 (+0.7)</td>
</tr>
<tr>
<td>Beta 2</td>
<td>9.0 (+1.5)</td>
<td>9.6 (+0.6)</td>
</tr>
<tr>
<td>Gamma</td>
<td>4.0 (+0.5)</td>
<td>3.4 (+0.3)</td>
</tr>
</tbody>
</table>

Values are reported as the % of the total protein for *n* = 35 animals per group.

Controls were untreated. Treated rats received 0.5% of the body weight/day of sesbania extracts for three days.

* = means are significantly different from the values obtained for control animals (P<0.05).
decreased while the alpha 2 fraction increased following sesbania treatment in the rat. Neither the beta nor the gamma fractions were significantly changed by sesbania treatment in the rat. It is important to note that while the absolute protein concentrations were not identical between control and sesbania treated animals, the relative proportions of each class were independent of the total protein concentration. These concentrations were about one half control values for both sesbania treated chickens and rats.

**Immunodiffusion:**

In both chickens and rats, the total protein and albumin concentrations were significantly (P<0.05) depressed by sesbania treatment (Table 18). The percentage decrease for both chickens and rats was greatest for the albumin fraction (being 42% and 52% of control values, respectively).

**Serum Enzymes:**

**PChE and AChE:**

The effects of sesbania treatment on serum PChE (Pseudocholinesterase) and AChE (Acetylcholinesterase) activities are given in Table 19. The activities of chicken serum PChE and AChE were depressed in sesbania treated birds, whereas in sesbania treated rats only serum PChE activity was depressed. Serum AChE activity in the rat remained unaltered by sesbania treatment (Table 19).
TABLE 18

The estimation of total protein and albumin concentrations in serum from control (untreated) and sesbania treated animals by radial immunodiffusion.

<table>
<thead>
<tr>
<th></th>
<th>CHICKENS</th>
<th></th>
<th></th>
<th>RATS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole Serum (mg/ml)</td>
<td>Albumin (mg/ml)</td>
<td>Whole Serum (mg/ml)</td>
<td>Albumin (mg/ml)</td>
</tr>
<tr>
<td>Control</td>
<td>3.50</td>
<td>1.76</td>
<td>Control</td>
<td>7.10</td>
</tr>
<tr>
<td>±0.10</td>
<td>±0.12</td>
<td>±0.10</td>
<td>±0.12</td>
<td>±0.10</td>
</tr>
<tr>
<td>Treated</td>
<td>2.14*</td>
<td>0.74*</td>
<td>Treated</td>
<td>5.20*</td>
</tr>
<tr>
<td>±0.05</td>
<td>±0.08</td>
<td>±0.09</td>
<td>±0.09</td>
<td>±0.09</td>
</tr>
</tbody>
</table>

Values are the means ± the SEM for n = 6 animals per group.

Control animals were untreated, while treated chickens received sesbania extracts at 1.0% and rats at 0.5% of body weight / day for three days.

* = means are significantly different from values obtained for control animals (P<0.05).
Table 19

Serum PChE, AChE, LDH, alkaline phosphatase and gamma glutamyl transpeptidase activities in chickens and rats dosed with *Sesbania drummondii*.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Rat Control</th>
<th>Rat Treated</th>
<th>Chicken Control</th>
<th>Chicken Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>PChE</td>
<td>1.47 +/- 0.02</td>
<td>1.05* +/- 0.01</td>
<td>1.40 +/- 0.00</td>
<td>0.80* +/- 0.00</td>
</tr>
<tr>
<td>AChE</td>
<td>0.59 +/- 0.03</td>
<td>0.64 +/- 0.05</td>
<td>0.56 +/- 0.01</td>
<td>0.32* +/- 0.00</td>
</tr>
<tr>
<td>LDH</td>
<td>128.00 +/- 0.74</td>
<td>27.20* +/- 0.39</td>
<td>169.37 +/- 1.36</td>
<td>142.17* +/- 1.99</td>
</tr>
<tr>
<td>APase</td>
<td>63.45 +/- 0.75</td>
<td>31.98* +/- 0.89</td>
<td>43.55 +/- 0.19</td>
<td>14.17* +/- 0.07</td>
</tr>
<tr>
<td>GGT</td>
<td>5.00 +/- 0.40</td>
<td>3.00* +/- 0.34</td>
<td>4.28 +/- 0.20</td>
<td>2.09* +/- 0.16</td>
</tr>
</tbody>
</table>

Values are the means +/- the SEM for n = 6 animals per group.

Control animals were untreated. Treated chickens received sesbania extracts at 1.0% of the body weight / day while rats were dosed at 0.5% of the body weight / day for three days.

PChE = serum pseudocholinesterase activity as nanomoles of substrate converted per minute per mg of protein.

AChE = acetylcholinesterase activity measured in an analogous manner as PChE using acetyl thiocholine iodine as substrate.

LDH = serum lactic dehydrogenase activity as nanomoles of substrate converted per minute per mg of protein.

APase = alkaline phosphatase activity expressed as nanomoles of para-nitrophenyl phosphate converted per minute per mg of protein.

GGT = gamma glutamyl transpeptidase activity expressed as nanomoles of substrate converted per minute per mg of protein.

* = means are significantly different from control values (P<0.05).
Phenobarbital pretreatment did not alter the activity of the PChE fraction in controls (Table 20) but did partially reverse the sesbania induced decrease in PChE activity. BNF (beta-naphthafлавone) treatment significantly (P<0.05) increased the activity of PChE compared with control animals as well as partially reversed the sesbania induced change in this enzyme (Table 20).

**Alkaline Phosphatase, Glutamyl Transpeptidase and Lactic Dehydrogenase:**

The serum activities of alkaline phosphatase (APase) and gamma glutamyl transpeptidase (GGT) were depressed in the serum of sesbania treated chickens (Table 19), but LDH activity was unchanged. In the rat, changes in serum lactic dehydrogenase, alkaline phosphatase and gamma glutamyl transpeptidase activities in response to sesbania treatment were depressed (Table 19).

**Liver Enzymes:**

**Liver Cholinesterase:**

Liver PChE activities in rat and chicken are given in Table 21. In the chicken, the activity of PChE was about equally distributed between the cytosolic and microsomal fractions. However, in the rat, the cytosolic activity represented the major fraction of the enzyme in the liver. Despite these species differences in distribution, sesbania was found to significantly (P<0.05) depress the activity of liver PChE in both cytosol and microsomes (Table 21).
TABLE 20

The influence of phenobarbital and beta-naphthaflavone (BNF) on circulating rat serum total (pseudo + true) cholinesterase activity.

<table>
<thead>
<tr>
<th></th>
<th>Total ChE (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.99 (+-0.00)</td>
</tr>
<tr>
<td>Sesbania</td>
<td>0.44* (+-0.00)</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>1.01~ (+-0.01)</td>
</tr>
<tr>
<td>Phenobarbital/Sesbania</td>
<td>0.76*~ (+-0.00)</td>
</tr>
<tr>
<td>BNF</td>
<td>1.40*~ (+-0.04)</td>
</tr>
<tr>
<td>BNF/Sesbania</td>
<td>1.13*~ (+-0.04)</td>
</tr>
</tbody>
</table>

Values are the means +- the SEM for n = 6 animals per group.

Control animals were untreated. Rats were dosed for 3 days with phenobarbital (80mg/kg) or BNF (40 mg/kg). Combination treatments were dosed for an additional two days with sesbania at 0.3% of the body weight / day.

Total ChE units are expressed as the nanomoles of butrylthiocholine iodine converted per minute per mg protein.

* = means are significantly different from values obtained for control animals (P<0.05).

~ = means are significantly different from values obtained for animals receiving sesbania extracts (P<0.05).
TABLE 21
Liver pseudocholinesterase activity in chickens and rats treated with 20K supernatant extract of *Sesbania drummondii*.

<table>
<thead>
<tr>
<th></th>
<th><strong>CHICKEN</strong></th>
<th></th>
<th><strong>RAT</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Microsomes</td>
<td>1.30</td>
<td>0.84*</td>
<td>0.05</td>
<td>0.03*</td>
</tr>
<tr>
<td></td>
<td>+-0.00</td>
<td>+-0.01</td>
<td>+-0.00</td>
<td>+-0.00</td>
</tr>
<tr>
<td>Cytosol</td>
<td>1.39</td>
<td>0.61*</td>
<td>1.35</td>
<td>1.10*</td>
</tr>
<tr>
<td></td>
<td>+-0.02</td>
<td>+-0.00</td>
<td>+-0.02</td>
<td>+-0.03</td>
</tr>
</tbody>
</table>

Values are the means +- the SEM for n = 19 animals for chickens and n = 6 animals for rats per group.

All animals were dosed for three days prior to sacrifice, chickens at 0.5% of the body weight and rats at 0.3% of the body weight/day.

PChE units are expressed as the nanomoles of substrate converted per mg of protein at 22C.

* = means are significantly different from values obtained for control animals (P<0.05).
Liver AChE activities in rat and chicken are shown in Table 22. AChE activity was similarly distributed in both chickens and rats and did not show any species variation in distribution (Table 22). Sesbania treatment produced decreases in AChE activities in both microsomes and cytosol for treated rats and chickens (P<0.05).

Liver Lactic Dehydrogenase:
Table 23 gives the data for liver LDH activities in both chickens and rats. For both species, sesbania treatments produced decreased microsomal activities (~50% of control values). The cytosolic fraction, representing the major portion of the enzyme activity, was unaffected by sesbania treatment (Table 23).

Liver Pyruvate Kinase:
The effects of sesbania treatment on the activities of pyruvate kinase in chickens and rats are shown in Table 24. Pyruvate kinase (PK), an allosteric liver enzyme in rats, associated with the metabolism of carbohydrates, is stimulated by the addition of fructose (bis) 1,6 diphosphate (FBP). The PK activities in the liver were approximately equally distributed between the cytosol and microsomes in both chickens and rats (Table 24). For the rat, sesbania treatment was found to remove about 50% of the activity. This depression remained both in the presence and absence of FBP stimulation though absolute enzyme activities were increased by FBP for both control and sesbania treated rats. In the chicken, the degree of stimulation by FBP was much less dramatic. In both control and treated chickens, FBP addition increased the overall
TABLE 22

Acetylcholinesterase activity in the livers of chickens and rats treated with 20K supernatant extracts of *Sesbania drummondii*.

<table>
<thead>
<tr>
<th></th>
<th>AChE Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>CHICKENS</em></td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Microsomes</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>+0.00</td>
</tr>
<tr>
<td>Cytosol</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>+0.03</td>
</tr>
</tbody>
</table>

Values are the means ± the SEM for *n* = 6 animals per group.

Control animals were untreated. Animals were dosed for three days at 1.0% of body weight for chickens, while rats were dosed at 0.5% of body weight /day.

Microsomes and cytosol were 100,000 *g* superantant (cytosol) and washed pellet (microsomes) fractions, respectively, from isolated liver samples.

* = means are significantly different from values obtained for control animals (P < 0.05).
### TABLE 23

Lactic dehydrogenase activity in the livers of chickens and rats treated with 20K supernatant extracts of *Sesbania drummondii*.

<table>
<thead>
<tr>
<th>Fraction</th>
<th><strong>CHICKENS</strong></th>
<th><strong>RATS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Microsomes</td>
<td>29.89</td>
<td>16.63*</td>
</tr>
<tr>
<td></td>
<td>+-0.43</td>
<td>+-1.53</td>
</tr>
<tr>
<td>Cytosol</td>
<td>173.08</td>
<td>165.71</td>
</tr>
<tr>
<td></td>
<td>+-4.73</td>
<td>+-10.60</td>
</tr>
</tbody>
</table>

Values are the means ± the SEM for n = 22 animals per group for chickens and n = 15 animals per group for rats.

Control animals were untreated. Chickens were dosed at 1.0% of the body weight / day for 3 days, while rats were also dosed for three days but at 0.3% of the body weight.

Microsomes and cytosol are the 100,000 x g supernatant and washed pellet fractions, respectively, obtained from isolated liver.

* = means are significantly different from values obtained for control animals (P<0.05).
### TABLE 24

Pyruvate kinase activity in the livers of chickens and rats undergoing treatment with sesbania extracts.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>PK (units)</th>
<th>CHICKEN</th>
<th>RAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Microsomes</td>
<td>0.072</td>
<td>0.056*</td>
<td>0.169</td>
</tr>
<tr>
<td></td>
<td>+-0.001</td>
<td>+-0.001</td>
<td>+-0.000</td>
</tr>
<tr>
<td>+FBP</td>
<td>0.083~</td>
<td>0.086~°</td>
<td>0.705~</td>
</tr>
<tr>
<td></td>
<td>+-0.001</td>
<td>+-0.003</td>
<td>+-0.006</td>
</tr>
<tr>
<td>Cytosol</td>
<td>0.056</td>
<td>0.042*</td>
<td>0.149</td>
</tr>
<tr>
<td></td>
<td>+-0.001</td>
<td>+-0.001</td>
<td>+-0.001</td>
</tr>
<tr>
<td>+FBP</td>
<td>0.076~</td>
<td>0.062*~</td>
<td>0.157~</td>
</tr>
<tr>
<td></td>
<td>+-0.000</td>
<td>+-0.000</td>
<td>+-0.001</td>
</tr>
</tbody>
</table>

Values are the means +- the SEM for n = 6 animals per group.

Control animals were untreated. Rats were dosed at 0.5% of the body weight/day while chickens received 1.0% of the body weight/day. Each group was dosed for three days.

Microsomes and cytosol are the 100,000 x g pellet and supernatant fractions, respectively of isolated liver.

FBP ~ is fructose (bis) 1,6 diphosphate, a known allosteric modifier of liver pyruvate kinase. It was used at the following concentration: 10 ul of 10mM solution per cuvette.

* = means are significantly different from values obtained for control animals (P<0.05).

~ = means are significantly different from pre FBP values (P<0.05).

° = means are significantly different in the ratio between control and activity following FBP treatment.
cytosolic activity by an equivalent amount, i.e. sesbania treatment had no effect on the ability of FBP to influence the cytosolic enzyme activity (Table 24). In the microsomal fraction FBP caused a slightly greater increase in sesbania treated animals than in control animals.

Liver Alkaline Phosphatase:

Table 25 shows the effects of sesbania treatment on the activities of liver alkaline phosphatase. Alkaline phosphatase (APase), an enzyme excreted into the serum via the biliary pathway, was found to be purely cytosolic in distribution in the livers of both chickens and rats (Table 25). Sesbania produced decreases in measurable activities in both animal species.

Liver Gamma Glutamyl Transpeptidase:

Liver gamma glutamyl transpeptidase activities and the influence of sesbania treatment on these activities are shown in Table 26. Gamma glutamyl transpeptidase (GGT) activities were examined in both chickens and rats. In treated chickens, no changes in the activity of this enzyme were noted in either the cytosolic or microsomal fractions (Table 26). In the rat, the majority of this enzyme was associated with the microsomes. This activity was severely reduced following sesbania treatment. The minor cytosolic activity was not affected by sesbania treatment in either species.
TABLE 25

Activity of liver alkaline phosphatase (APase) in chickens and rats undergoing sesbania treatment.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>CHICKEN Alkaline Phosphatase</th>
<th>RAT Alkaline Phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Cytosol</td>
<td>2.246</td>
<td>0.812*</td>
</tr>
<tr>
<td></td>
<td>+/-0.036</td>
<td>+/-0.003</td>
</tr>
<tr>
<td>Microsome</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

Values are the means +/- the SEM for n = 26 chickens and n = 12 rats for each group.

Control animals were untreated. Treated animals were dosed for three days, chickens are 1.0% of the body weight and rats at 0.5% of the body weight/day.

APase units are expressed as nanomoles of substrate converted per minute per mg of protein at 22°C.

* = means are significantly different from values obtained for control animals (P<0.05).
TABLE 26
Activities of gamma glutamyl transpeptidase (GGT) in the livers of chickens and rats receiving sesbania extracts.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Glutamyl Transpeptidase (CHICKEN)</th>
<th>Glutamyl Transpeptidase (RAT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Cytosol</td>
<td>9.247</td>
<td>9.306</td>
</tr>
<tr>
<td></td>
<td>+/-0.042</td>
<td>+/-0.061</td>
</tr>
<tr>
<td>Microsome</td>
<td>2.225</td>
<td>2.489</td>
</tr>
<tr>
<td></td>
<td>+/-0.042</td>
<td>+/-0.061</td>
</tr>
</tbody>
</table>

Values are the means +/- the SEM for n = 6 animals per group.

Control animals were untreated. All treated animals were dosed for three days, chickens at 1.0% of the body weight and rats at 0.3% of the body weight/day.

GGT units are expressed as nanomoles of substrate converted to product per minute per mg of protein.

* = means are significantly different from values obtained for control animals (P<0.05).
Glutathione-S-Transfersases:

In the mature chicken (6-9 weeks of age), microsomal ligandin (GST-I) activity was increased by sesbania (~200% of control) and phenobarbital treatments (~200% of control) as shown in Table 27. The combination treatment with phenobarbital and sesbania depressed this activity below control values (less than 25% of control). BNF treatment did not alter the activity of this microsomal isozyme. The combination treatment of BNF and sesbania reduced the activity of this isozyme in isolated microsomes (less than 25% of control).

In the mature chicken cytosol, sesbania treatment increased the activity of GST-I (387% of control). Phenobarbital treatment produced no change in enzyme activity, while the combination treatment of phenobarbital and sesbania also increased the activity of GST-I (198% of control). This increase by phenobarbital and sesbania treatment was less than the increase produced by sesbania alone. BNF or BNF and sesbania treatments produced no change in the enzyme activity.

In the immature chicken (less than 14 days old), the activity of microsomal GST-I was increased by phenobarbital and sesbania or BNF treatment alone (Table 27). All other treatments produced no effects on enzyme activity. The cytosolic activity of GST-I was increased 110% by sesbania treatment, 1389% by phenobarbital treatment and 1789% by phenobarbital and sesbania treatment. This increase by phenobarbital and sesbania combination treatment was simple addition of the two individual treatments. BNF treatment and
TABLE 27
Activities of GST-1 (ligandin, DNCB) in chicken following sesbania and/or phenobarbital and/or BNF treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mature Microsome</th>
<th>Mature Cytosol</th>
<th>Immature Microsome</th>
<th>Immature Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0114 ± 0.0029</td>
<td>0.0061 ± 0.0009</td>
<td>0.0022* ± 0.0002</td>
<td>0.0063~ ± 0.0012</td>
</tr>
<tr>
<td>Sesbania</td>
<td>0.0260* ± 0.0047</td>
<td>0.0236* ± 0.0070</td>
<td>0.0032* ± 0.0004</td>
<td>0.0132*~ ± 0.0008</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.0283* ± 0.0052</td>
<td>0.0063~ ± 0.0008</td>
<td>0.0036* ± 0.0006</td>
<td>0.0938*~ ± 0.0194</td>
</tr>
<tr>
<td>Phenobarbital +</td>
<td>0.0024*Δ ± 0.0013</td>
<td>0.0121* ± 0.0018</td>
<td>0.0077* ± 0.0017</td>
<td>0.1190*~ ± 0.0275</td>
</tr>
<tr>
<td>BNF</td>
<td>0.0060 Δ ± 0.0002</td>
<td>0.0090 ± 0.0013</td>
<td>0.0048* ± 0.0003</td>
<td>0.0068 ± 0.0012</td>
</tr>
<tr>
<td>BNF + Sesbania</td>
<td>0.0020*Δ ± 0.0004</td>
<td>0.0053~ ± 0.0011</td>
<td>0.0045 ± 0.0058</td>
<td>0.0087 ± 0.0014</td>
</tr>
</tbody>
</table>

Values are the mean ± the SEM for n = 6 animals per group.

Controls were untreated. Chickens were dosed with 80 mg/kg of phenobarbital or 40 mg/kg of BNF for three days followed by 1.0% of the body weight/day of a 20K sesbania extract for 2 days prior to sacrifice.

* = means are significantly different from values obtained for control animals (P<0.05).

~ = means of cytosolic values are significantly different from values obtained for microsomal fraction (P<0.05).

° = means of immature values are significantly different from values obtained for mature chickens (P<0.05).

Δ = means are significantly different from values obtained for sesbania treated animals (P<0.05).
the combination treatment showed little effect on cytosolic GST-I activity.

The effects of sesbania on GST-II activities are given in Table 28. None of the treatment regimens gave values which were significantly different from control values.

The effects of sesbania and other treatments on GST-III activities are given in Table 29. Mature chicken microsomal and immature chick cytosolic fraction receiving BNF treatment showed increased GST III activity. All other treatments were without effect on GST-III activities.

The activity of mature chicken microsomal GST-IV activity was increased by sesbania, phenobarbital and the combination of BNF and sesbania (Table 30). The other treatments produced no effects. This enzyme activity in mature chicken cytosol was increased by BNF and sesbania combination treatments alone. No other changes were noted. For the immature chicken only, all treatments significantly lowered the microsomal activities (sesbania, 3.3% of control; phenobarbital, 1.90% of control, phenobarbital and sesbania, 13.5% of control; BNF, 0.3% of control and BNF and sesbania, 1.1% of control). In the immature chicken cytosolic fraction, sesbania (543% of control), phenobarbital and sesbania (286% of control) and BNF (557% of control) treatments increased GST-IV activity.

Values for rat GST-I microsomal and cytosolic activities are shown in Table 31. The mature rat microsomal GST-I activity was depressed by BNF. The combination treatment of BNF and sesbania failed to reverse the BNF inhibition. Other treatments produced activities which were similar to control values. Mature rat
### TABLE 28
Activities of GST-II (DCNB) in chicken following sesbania and/or phenobarbital and/or BNF treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mature</th>
<th>Immature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microsome</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Control</td>
<td>0.0104</td>
<td>0.0108</td>
</tr>
<tr>
<td></td>
<td>+0.0029</td>
<td>+0.0038</td>
</tr>
<tr>
<td>Sesbania</td>
<td>0.0105</td>
<td>0.0083</td>
</tr>
<tr>
<td></td>
<td>+0.0022</td>
<td>+0.0022</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.0088</td>
<td>0.0054</td>
</tr>
<tr>
<td></td>
<td>+0.0028</td>
<td>+0.0009</td>
</tr>
<tr>
<td>Pheno + Sesbania</td>
<td>0.0131</td>
<td>0.0079</td>
</tr>
<tr>
<td></td>
<td>+0.0044</td>
<td>+0.0013</td>
</tr>
<tr>
<td>BNF</td>
<td>0.0083</td>
<td>0.0012~</td>
</tr>
<tr>
<td></td>
<td>+0.0010</td>
<td>+0.0002</td>
</tr>
<tr>
<td>BNF + Sesbania</td>
<td>0.0090</td>
<td>0.0019</td>
</tr>
<tr>
<td></td>
<td>+0.0095</td>
<td>+0.0004</td>
</tr>
</tbody>
</table>

* = means are significantly different from the values obtained for control animals (P<0.05).

~ = means for the cytosolic values are significantly different from values obtained for microsomal fractions (P<0.05).

° = means for immature chicken are significantly different from values obtained for mature chicken (P<0.05).
TABLE 29

Activities of GST-III (NPA) in chickens treated with sesbania and/or phenobarbital and/or BNF.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mature Microsome</th>
<th>Immature Microsome</th>
<th>Mature Cytosol</th>
<th>Immature Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.05 +/- 9.20</td>
<td>71.34 +/- 5.22</td>
<td>23.40 +/- 3.37</td>
<td>29.70 +/- 1.93</td>
</tr>
<tr>
<td>Sesbania</td>
<td>79.48 +/- 16.87</td>
<td>76.54 +/- 13.80</td>
<td>18.11 +/- 2.88</td>
<td>28.71 +/- 3.92</td>
</tr>
<tr>
<td>Pheno-barbital</td>
<td>41.84 +/- 5.25</td>
<td>126.78° +/- 28.24</td>
<td>15.22 +/- 2.03</td>
<td>25.20° +/- 5.56</td>
</tr>
<tr>
<td>Pheno +</td>
<td>155.13 +/- 53.81</td>
<td>89.19 +/- 20.31</td>
<td>22.33 +/- 3.97</td>
<td>22.12 +/- 4.70</td>
</tr>
<tr>
<td>Sesbania</td>
<td>80.85 +/- 3.85</td>
<td>87.09 +/- 9.45</td>
<td>13.32 +/- 0.68</td>
<td>31.37 +/- 1.37</td>
</tr>
</tbody>
</table>

* = means are significantly different from values obtained for control animals (P<0.05).

~ = means for cytosolic fractions are significantly different from values obtained for microsomal fractions (P<0.05).

° = means for immature animals are significantly different from values obtained for mature chicken (P<0.05).
TABLE 30

Activities of GST-IV (4BD) in chicken following sesbania and / or phenobarbital and / or BNF treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mature Microsome</th>
<th>Cytosol</th>
<th>Immature Microsome</th>
<th>Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0018</td>
<td>0.0005~</td>
<td>0.1062°</td>
<td>0.0007~</td>
</tr>
<tr>
<td></td>
<td>+0.0003</td>
<td>+0.0001</td>
<td>+0.0249</td>
<td>+0.0001</td>
</tr>
<tr>
<td>Sesbania</td>
<td>0.0070*</td>
<td>0.0006~</td>
<td>0.0035*</td>
<td>0.0038°</td>
</tr>
<tr>
<td></td>
<td>+0.0028</td>
<td>+0.0001</td>
<td>+0.0006</td>
<td>+0.0008</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.0061*</td>
<td>0.0004~</td>
<td>0.0020*</td>
<td>0.0007~</td>
</tr>
<tr>
<td></td>
<td>+0.0015</td>
<td>+0.0000</td>
<td>+0.0004</td>
<td>+0.0001</td>
</tr>
<tr>
<td>Pheno + Sesbania</td>
<td>0.0048</td>
<td>0.0004~</td>
<td>0.0143*</td>
<td>0.0020°</td>
</tr>
<tr>
<td></td>
<td>+0.0018</td>
<td>+0.0001</td>
<td>+0.0033</td>
<td>+0.0003</td>
</tr>
<tr>
<td>BNF</td>
<td>0.0030</td>
<td>0.0006~</td>
<td>0.0003°</td>
<td>0.0039°</td>
</tr>
<tr>
<td></td>
<td>+0.0004</td>
<td>+0.0000</td>
<td>+0.0000</td>
<td>+0.0008</td>
</tr>
<tr>
<td>BNF + Sesbania</td>
<td>0.0073*</td>
<td>0.0039*</td>
<td>0.0012°</td>
<td>0.0006°</td>
</tr>
<tr>
<td></td>
<td>+0.0016</td>
<td>+0.0008</td>
<td>+0.0002</td>
<td>+0.0001</td>
</tr>
</tbody>
</table>

* = means are significantly different from values obtained for control animals (P<0.05).

~ = cytosolic means are significantly different from values obtained for microsomal fractions in the chicken (P<0.05).

° = means obtained for immature animals are significantly different from values obtained for mature chickens (P<0.05).
TABLE 31
Activities of GST-I (ligandin, DNCB) in rats following sesbania, and / or phenobarbital and / or BNF treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mature Microsome</th>
<th>Mature Cytosol</th>
<th>Immature Microsome</th>
<th>Immature Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0117 ± 0.0024</td>
<td>0.0112</td>
<td>0.0014* ± 0.0002</td>
<td>0.0063~ ± 0.0014</td>
</tr>
<tr>
<td>Sesbania</td>
<td>0.0055 ± 0.0193</td>
<td>0.0074 ± 0.0023</td>
<td>0.0090* ± 0.0010</td>
<td>0.0163*~ ± 0.0021</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.0097 ± 0.0018</td>
<td>0.0121</td>
<td>0.0036** ± 0.0008</td>
<td>0.0014*~ ± 0.0001</td>
</tr>
<tr>
<td>Pheno + Sesbania</td>
<td>0.0140 ± 0.0021</td>
<td>0.0136</td>
<td>0.0067** ± 0.0007</td>
<td>0.0103</td>
</tr>
<tr>
<td>BNF</td>
<td>0.0014* ± 0.0001</td>
<td>0.0066~ ± 0.0014</td>
<td>0.0081** ± 0.0017</td>
<td>0.0179*~ ± 0.0002</td>
</tr>
<tr>
<td>BNF + Sesbania</td>
<td>0.0011* ± 0.0000</td>
<td>0.0025*~ ± 0.0002</td>
<td>0.0068** ± 0.0005</td>
<td>0.0321*~ ± 0.0056</td>
</tr>
</tbody>
</table>

* = means are significantly different from values obtained for control rats (P<0.05).

~ = cytosolic values are significantly different from values obtained for microsomal fractions (P<0.05).

• = immature values are significantly different from values obtained for mature rats (P<0.05).
cytosolic activity was depressed by BNF and sesbania treatment, even though neither individual treatment had shown an effect. For the immature rat microsome, all treatments increased GST-I activity. For immature rat cytosolic GST-I activities, sesbania alone or in combination produced increased values. BNF treatment also produced increased activity, whereas, phenobarbital treatment decreased GST-I activity. In all combinations treatments the effects of sesbania were additive to other treatments.

A significant decrease in activity produced by BNF and BNF and sesbania treatments on mature rat microsomal GST-II can be seen in Table 32. No other treatment caused any change in enzyme activity. The cytosolic activities were depressed by all treatments in the mature rat. This effect was least pronounced for the combination treatment of phenobarbital plus sesbania which was not significantly (P>0.05) below values obtained for control animals.

For the immature rat, sesbania, sesbania and phenobarbital, BNF or BNF and sesbania treatments significantly (P<0.05) increased the activity of microsomal GST-II. Phenobarbital treatment alone was without effect. In immature rat cytosol, the combination treatment of phenobarbital and sesbania gave GST-II activities which were not significantly different from control values, although this lack of significance might be due to the large standard error obtained. Phenobarbital treatment depressed this activity. All other treatments (i.e. sesbania, BNF or BNF and sesbania) significantly increased the activity of GST-II in immature rat cytosol.
TABLE 32

Activities of GST-II (DCNB) in rats treated with sesbania and / or phenobarbital and / or BNF.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mature Microsome</th>
<th>Cytosol</th>
<th>Immature Microsome</th>
<th>Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0024 ± 0.0003</td>
<td>0.0193 ± 0.0024</td>
<td>0.0017 ± 0.0013</td>
<td>0.0060 ± 0.0011</td>
</tr>
<tr>
<td>Sesbania</td>
<td>0.0014 ± 0.0002</td>
<td>0.0101± 0.0016</td>
<td>0.0097± 0.0013</td>
<td>0.0183± 0.0029</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.0031 ± 0.0004</td>
<td>0.0108± 0.0019</td>
<td>0.0033 ± 0.0007</td>
<td>0.0016± 0.0005</td>
</tr>
<tr>
<td>Pheno + Sesbania</td>
<td>0.0021 ± 0.0048</td>
<td>0.0122± 0.0017</td>
<td>0.0087± 0.0008</td>
<td>0.0093 ± 0.0025</td>
</tr>
<tr>
<td>BNF</td>
<td>0.0007* ± 0.0001</td>
<td>0.0010* ± 0.0000</td>
<td>0.0088* ± 0.0019</td>
<td>0.0182* ± 0.0023</td>
</tr>
<tr>
<td>BNF + Sesbania</td>
<td>0.0002* ± 0.0000</td>
<td>0.0021*± 0.0001</td>
<td>0.0083* ± 0.0012</td>
<td>0.0305* ± 0.0061</td>
</tr>
</tbody>
</table>

* = means are significantly different from values obtained for control animals (P<0.05).

~ = cytosolic values are significantly different from values obtained for microsomal fractions (P<0.05).

° = immature means are significantly different from values obtained for mature rats (P<0.05).
The effects of sesbania and / or phenobarbital and / or BNF treatments on rat GST-III activities are shown in Table 33. Sesbania, phenobarbital or the combination treatment (sesbania and phenobarbital) slightly increased the activities of mature rat microsomal GST-III. BNF alone caused a significant decline in activity while the combination of BNF and sesbania was not significantly different from control values. The cytosolic enzyme activity was unchanged by any treatment. As with the mature rat, immature microsomal activities of GST-III were increased by sesbania and phenobarbital. In contrast with the mature values, however, treatment with phenobarbital and sesbania gave values which were not significantly above control values. Whereas BNF in the mature rat caused a decline in GST-III activity, BNF or BNF and sesbania treatments increased immature microsomal GST-III. No treatment effects were observed for mature cytosolic values, but phenobarbital or phenobarbital and sesbania caused significant declines in the activity of this isozyme in immature rats. BNF treatment caused a significant increase in immature cytosolic GST-III. The combination treatment was neither additive nor synergistic.

Rat GST-IV activities are shown in Table 34. Phenobarbital, BNF or BNF and sesbania treatments caused significant declines in the mature rat microsomal GST-IV activities. Sesbania treatment had no effect. The cytosolic isozyme was unaffected by any treatment. In immature rats, sesbania treatment had no effect on enzyme activity, but phenobarbital treatment caused significant increases in activities whereas the combination treatment
### TABLE 33

Activities of GST-III (NPA) in rats treated with sesbania and / or phenobarbital and / or BNF.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mature Microsome</th>
<th>Cytosol</th>
<th>Immature Microsome</th>
<th>Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>110.89 ± 4.99</td>
<td>12.47~  ± 5.85</td>
<td>16.52° ± 0.97</td>
<td>69.17± 6.34</td>
</tr>
<tr>
<td>Sesbania</td>
<td>128.67* ± 2.94</td>
<td>14.88~ ± 1.50</td>
<td>27.02° ± 1.73</td>
<td>84.63~14.05</td>
</tr>
<tr>
<td>Pheno-</td>
<td>124.72* ± 0.59</td>
<td>21.53~ ± 3.65</td>
<td>20.70° ± 0.65</td>
<td>34.43°4.36</td>
</tr>
<tr>
<td>barbital</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pheno +</td>
<td>130.03* ± 3.72</td>
<td>17.76~ ± 2.60</td>
<td>15.38° ± 1.00</td>
<td>34.76°4.06</td>
</tr>
<tr>
<td>Sesbania + BNF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNF</td>
<td>86.50* ± 0.39</td>
<td>31.02~ ± 5.39</td>
<td>23.90° ± 0.70</td>
<td>86.53°0.06</td>
</tr>
<tr>
<td>BNF +</td>
<td>103.21 ± 10.32</td>
<td>14.07~ ± 1.30</td>
<td>21.28° ± 0.12</td>
<td>75.78°0.40</td>
</tr>
</tbody>
</table>

* = means are significantly different from values obtained for control animals (P<0.05).

~ = cytosolic means are significantly different from microsomal fractions in the rat (P<0.05).

° = immature values are significantly different from values obtained for mature rats (P<0.05).
TABLE 34

Activities of GST-IV (4BD) in rats treated with sesbania and / or phenobarbital and / or BNF.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mature Microsome</th>
<th>Mature Cytosol</th>
<th>Immature Microsome</th>
<th>Immature Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0044</td>
<td>0.0003~</td>
<td>0.0002°</td>
<td>0.0014~°</td>
</tr>
<tr>
<td></td>
<td>±0.0007</td>
<td>±0.0000</td>
<td>±0.0000</td>
<td>±0.0001</td>
</tr>
<tr>
<td>Sesbania</td>
<td>0.0030</td>
<td>0.0004~</td>
<td>0.0001°</td>
<td>0.0013~°</td>
</tr>
<tr>
<td></td>
<td>±0.0067</td>
<td>±0.0001</td>
<td>±0.0000</td>
<td>±0.0002</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.0008*</td>
<td>0.0006</td>
<td>0.0001</td>
<td>0.0004*~°</td>
</tr>
<tr>
<td></td>
<td>±0.0001</td>
<td>±0.0000</td>
<td>±0.0000</td>
<td>±0.0009</td>
</tr>
<tr>
<td>Phenob+Sesb</td>
<td>0.0028</td>
<td>0.0005~</td>
<td>0.0005</td>
<td>0.0004*</td>
</tr>
<tr>
<td></td>
<td>±0.0006</td>
<td>±0.0001</td>
<td>±0.0001</td>
<td>±0.001</td>
</tr>
<tr>
<td>BNF</td>
<td>0.0007*</td>
<td>0.0002</td>
<td>0.0004</td>
<td>0.0014~°</td>
</tr>
<tr>
<td></td>
<td>±0.0000</td>
<td>±0.0000</td>
<td>±0.0001</td>
<td>±0.0001</td>
</tr>
<tr>
<td>BNF+Sesb</td>
<td>0.0007*</td>
<td>0.0002</td>
<td>0.0008</td>
<td>0.0013°</td>
</tr>
<tr>
<td></td>
<td>±0.0001</td>
<td>±0.0000</td>
<td>±0.0018</td>
<td>±0.0001</td>
</tr>
</tbody>
</table>

* = means are significantly different from values obtained for control values (P<0.05).

~ = cytosolic fractions are significantly different from microsomal fractions in the rat (P<0.05).

° = immature values are significantly different from values obtained for mature rats (P<0.05).
(phenobarbital and sesbania) lowered activities. BNF or BNF and sesbania had no effect on immature rat cytosolic activity GST-IV.

**Cytochrome P-450 Monooxygenase:**

In a preliminary experiment with two animals per group, sesbania treatment was found to increase the concentration of cytochrome P-450 in chickens (Table 35) in agreement with previous observations by Banton et al., 1987. The sensitivity of the scan did not permit differentiation between cytochromes P-450 and P-448.

**Liver Gluthathione Peroxidase:**

Gluthathione peroxidase is an enzyme which helps to maintain membrane stability, especially by reducing lipid peroxidation. Sesbania treatment reduced chicken microsomal activity by 50%. Both BNF and phenobarbital increased the activity in chicken microsomes. These increases were not reduced by sesbania pretreatment (Table 36). In the rat, sesbania had no effect on this enzyme activity. Only phenobarbital increased microsomal glutathione peroxidase activity. This effect was slightly reduced when sesbania was combined with the initial phenobarbital pretreatment (Table 36). BNF had no effect on enzyme activity.

The cytosolic activities of glutathione peroxidase were increased in both sesbania treated chickens and rats (Table 37). Similarly, both BNF and phenobarbital treatments increased the activity of this enzyme. The effect of BNF and sesbania treatment caused a synergistic increase in activity in the chicken but additive
TABLE 35

The effect of sesbania on the concentration of cytochrome P-450 in chicken liver.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P-450 nmole/mg microsomal protein</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.256</td>
<td>----</td>
</tr>
<tr>
<td>Treated</td>
<td>0.732</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Values are the mean of a duplicate liver sample for two animals per group.
TABLE 36

Microsomal glutathione peroxidase activities in chicken and rats receiving either sesbania and/or phenobarbital and/or BNF treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GLUTATHIONE PEROXIDASE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHICKEN</td>
</tr>
<tr>
<td>Control</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>+0.04</td>
</tr>
<tr>
<td>Sesbania</td>
<td>0.23*</td>
</tr>
<tr>
<td></td>
<td>+0.01</td>
</tr>
<tr>
<td>BNF</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>+0.00</td>
</tr>
<tr>
<td>BNF + Sesbania</td>
<td>1.29*</td>
</tr>
<tr>
<td></td>
<td>+0.00</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>2.99*</td>
</tr>
<tr>
<td></td>
<td>+0.01</td>
</tr>
<tr>
<td>Pheno + Sesbania</td>
<td>3.01*</td>
</tr>
<tr>
<td></td>
<td>+0.01</td>
</tr>
</tbody>
</table>

Values are the means ± the SEM for n = 6 animals per group.

Control animals were untreated. Phenobarbital concentration was 80 mg/kg for 3 days, while BNF was used at a concentration of 40 mg/kg alone or followed by sesbania (1.0% body weight/day for chickens and 0.5% body weight/day for rats) for 2 days.

* = Means are significantly different from values obtained for control animals (P<0.05).

° = Means are significantly lower than phenobarbital treatment alone (P<0.05).
TABLE 37
Cytosolic glutathione peroxidase activities in chickens and rats receiving sesbania and/or phenobarbital and/or BNF treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glutathione Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>CHICKEN</strong></td>
</tr>
<tr>
<td>Control</td>
<td>2.99</td>
</tr>
<tr>
<td></td>
<td>+-0.01</td>
</tr>
<tr>
<td>Sesbania</td>
<td>5.31*</td>
</tr>
<tr>
<td></td>
<td>+-0.01</td>
</tr>
<tr>
<td>BNF</td>
<td>5.59*</td>
</tr>
<tr>
<td></td>
<td>+-0.21</td>
</tr>
<tr>
<td>BNF + Sesbania</td>
<td>14.37*</td>
</tr>
<tr>
<td></td>
<td>+- 0.47</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>11.31*</td>
</tr>
<tr>
<td></td>
<td>+- 0.44</td>
</tr>
<tr>
<td>Pheno+ Sesbania</td>
<td>13.50*</td>
</tr>
<tr>
<td></td>
<td>+-0.36</td>
</tr>
</tbody>
</table>

Values are the means +- the SEM for n = 6 animals per group.

* = means are significantly different from values obtained for control animals (P<0.05).
in the rat (Table 37). The effect of phenobarbital and sesbania treatment was less than additive in both chickens and rats.

**Liver Glucose-6-Phosphate Dehydrogenase:**

Glucose-6-phosphate dehydrogenase is a major enzyme in the pathway responsible for supplying the cell with reducing power in the form of NADPH. In chickens, sesbania produced no changes in either microsomal or cytosolic activities of this enzyme (Table 38). Sesbania treatment decreased rat microsomal activity but had no effect on cytosolic activity (Table 38).

**Sulfhydryl Group:**

Reduced glutathione is a cofactor in reactions mediated by glutathione peroxidase and other important xenobiotic metabolizing enzyme systems. Sulfhydryl availability was unchanged in animals undergoing sesbania treatment (Table 39).
TABLE 38

Glucose-6-phosphate dehydrogenase activity in the livers of chickens and rats treated with sesbania.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>CHICKEN</th>
<th></th>
<th>RAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>Microsomes</td>
<td>0.08</td>
<td>0.10</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>+0.01</td>
<td>+0.00</td>
<td>+0.02</td>
</tr>
<tr>
<td>Cytosol</td>
<td>0.08</td>
<td>0.10</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>+0.01</td>
<td>+0.02</td>
<td>+0.01</td>
</tr>
</tbody>
</table>

Values are the means ± the SEM for n = 6 animals per group.

Controls were untreated. Rats were dosed at 0.5% of the body weight/day while chickens received 1.0% of the body weight/day for three days of a 20K sesbania extract.

* = means are significantly different from the values obtained for control animals (P<0.05).
TABLE 39

Sulphhydryl group availability as estimated by reduced glutathione concentration (GSH) in the livers of sesbania treated chickens and rats.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>CHICKEN</th>
<th></th>
<th>RAT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Microsomes</td>
<td>0.46</td>
<td>0.51</td>
<td>0.65</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.01</td>
</tr>
<tr>
<td>Cytosol</td>
<td>2.76</td>
<td>2.81</td>
<td>2.15</td>
<td>1.99</td>
</tr>
<tr>
<td></td>
<td>+0.04</td>
<td>+0.07</td>
<td>+0.04</td>
<td>+0.01</td>
</tr>
</tbody>
</table>

Values are the means ± the SEM for n = 6 animals per group.

Controls were untreated. Treated rats were dosed at a rate of 0.5% of the body weight / day and chickens at 1.0% of the body weight / day for three days.

* = means are significantly different from values obtained for control animals (P<0.05).
DISCUSSION

This study had two major objectives. The first was the initial isolation and identification of the toxic principle(s) of *Sesbania drummondii*, and the second was to study the mechanism(s) of action of these toxin(s) in hepatic tissue. The first section of this treatise described some of the physical characteristics of the toxic principle(s). Though complete isolation was not achieved, relevant information regarding temperature, pH and general lability of the toxin(s) was obtained. An important contribution of this work was the accumulation of evidence that supported the postulate that there is more than one major toxin in *Sesbania drummondii*.

The separation of toxicity from *Sesbania drummondii* seeds into different pH and molecular weight fractions lent credence to the hypothesis that more than one toxin is necessary to account for all the physiological effects observed with sesbania poisoning. Heat treatment of *Sesbania drummondii* extracts completely eliminated the weight loss produced in treated animals, even though decreases in circulating serum protein concentrations and lower PChE activities were still observed. This indicated that the toxin responsible for weight loss was heat labile. In contrast, acid treatment of extracts (pH 2 supernatant fractions) caused enhanced weight loss in treated rats when compared with animals receiving the untreated 20K extract. This increase in weight loss was not accompanied by increased mortality. Extracts of *Sesbania*
*drummondii* treated with alkali showed the reverse pattern for weight loss and mortality. Alkali treatment increased the mortality of treated animals (pH 8 supernatant fraction) while decreasing weight loss. Above pH 10, extract toxicity declined precipitously.

The seed portion of the plant is highly toxic (Flory and Hébert, 1984). Freeze-thawing of an aqueous filtrate of ground sesbania seeds disrupted the seed material sufficiently to release a previously non-dialyzable toxin from the seeds. This preparation produced a larger decrease in both serum protein concentration and PChE activity in treated animals when compared with animals dosed with unfrozen 20K extract, although it produced less weight loss. There is the possibility that the toxin responsible for weight loss is cold labile. The results also suggested that in its native state the toxin responsible for decreases in serum protein concentration and PChE activity was part of a larger molecular weight complex, perhaps non-covalently associated with non-dialyzable material.

The loss in toxicity following passage of 20K extracts through agarose, Sephadex™ G-25 or Con A-Sepharose indicated that one or more of the sesbania toxins could either be a lectin or had the capacity of interacting with carbohydrates. The inability to elute these toxic agents with alpha-methyl-glucoside, as well as the apparent small molecular weight of the toxins (<1000MW using hollow fiber and Amicon ultrafiltration fractionation), makes a protein-lectin unlikely. It is probable that electrostatic interactions between these matrices led to irreversible binding of sesbania toxic principles to the matrices. This phenomenon has been
well documented and is prevalent for the tightly cross-linked dextrans as well as for small aromatic compounds (Janson, 1966).

One of the purported toxins from *Sesbania drummondii* is sesbanimide. It is a small (MW 326), dialyzable, heat and alkali labile compound (Powell *et al.*, 1984). Chickens dosed with sesbanimide preparations lost weight, showed reduced circulating serum protein concentrations and a dose-related decrease in PChE activities typical of experimentally induced sesbania intoxication. Since sesbanimide had been previously isolated from the seeds of *Sesbania drummondii* (Powell *et al.*, 1984), there was no reason to assume that sesbanimide would not be present in our extracts. Sesbanimide was tentatively detected by both HPLC and thin layer chromatography. The major peak of the sesbanimide preparation had a retention time of 9.56 minutes and a minor peak at 7.40 minutes. This latter peak corresponded quite closely with the major peak in a partially purified sesbania extract (7.35 minutes). Pure sesbanimide was more toxic when dosed on the basis of equivalent peak heights than partially purified extract. Evidence that the extracts contained sesbanimide was obtained by thin layer chromatography on charring of silica plates with concentrated H$_2$SO$_4$.

If the mechanism of action of sesbanimide is, as proposed by Powell *et al* (1984), mediated by inhibition of protein synthesis, then similarities between sesbanimide poisoning and other protein synthesis inhibitors should be observed. Inhibition of protein synthesis has been postulated as the probable mechanism for the antineoplastic activity of sesbanimide. Sesbanimide shares a
structural similarity to the protein synthesis inhibitor, cycloheximide.

When cycloheximide, a glutaride type antibiotic, was tested for its ability to induce sesbania-like intoxication, it was found that many of the clinical signs were similar but not identical to those observed in birds and rats treated with sesbania extracts. Since cycloheximide is an inhibitor of protein synthesis, one would expect to observe decreases in both serum protein concentrations and PChE activities with this treatment. Such decreases were noted in cycloheximde treated animals. Although the results obtained with sesbania extracts were similar to those observed for animals receiving cycloheximide, there were important differences. The decrease in PChE activity associated with cycloheximide was independent of dose, whereas in sesbania treated rats, serum PChE activity was dose-related until at least day three of dosing (Table 11B). Decreases in serum protein concentrations, as a result of treatment, were greater for sesbania than for cycloheximide treatments. Due to the dose relationship, the effects of sesbania extracts on the activity of PChE in treated animals were more specific than would have been produced by a generalized protein synthesis inhibitor. The strongest evidence for differences between sesbania extract and cycloheximide treatments came from histological and electron microscopic examinations. With cycloheximide treatment, liver damage was evident but no damage was observed in histological sections from rats dosed with sesbania extracts. In those animals undergoing cycloheximide treatment, severe vacuolization of the liver was evident. This was
confirmed by thin section analysis. Cycloheximide treatment produced a complete change in the liver architecture which was not observed in sesbania treated rats.

Additional evidence for the presence of more than one toxin in crude sesbania extracts came from the observation that heating the extracts resulted in a complete loss of the ability of the extracts to induce weight loss. However, the heated extracts were still effective in producing decreases in both serum protein concentration and PChE activity. Also, the slight hemolysis that was observed was also not consistent with the effects of a protein synthesis inhibitor. Hemolysis had not been observed in animals treated with the known protein synthesis inhibitor, cycloheximide. It thus appeared that if sesbanimide's mode of action were the inhibition of protein synthesis, sesbanimide could not be the major toxic principle even though other toxins have not yet been isolated.

Additional physical evidence exists for the separation of sesbanimide toxicity from the major toxicity of crude sesbania extracts. Sesbanimide is not extractable into aqueous fractions, whereas most of the toxicity of crude sesbania preparations is extractable into aqueous fractions.

The most consistent finding in sesbania intoxication was severe gastric enteritis in both treated chickens and rats. This observation, coupled with the presence of frequent petechial hemorrhages, suggested that a neutral saponin, such as that isolated by Robey (1925), could be a causative agent in sesbania poisoning. Saponins are extremely irritating substances which can cause hemolysis. Hemolysis was noted as a frequent finding in the
sera obtained from sesbania treated animals. Hematocrits were, however, significantly lowered only in treated chickens (Table 15), although there was a statistically non-significant decline in the sera from sesbania treated rats. Despite the fact that the chicken red blood cell is nucleated and retains nuclear metabolic function, it apparently lacked the ability to limit sesbania induced hemolysis. The clinical observation of hemolysis supported the hypothesis that a saponotoxin may be present in crude extracts of *Sesbania drummondii*. It is also obvious that not all of the pathological findings of sesbania intoxication can be accounted for by the presence of a saponotoxin. Rats were found to be more sensitive than chickens to the actions of sesbania extracts, especially in their inability to tolerate higher doses. However, rat hematocrits remained unchanged, mitigating against the role of a saponotoxin as the major toxin in the pathogenesis of sesbania intoxication in this species.

The presence of a smooth muscle inhibitor in sesbania has been implicated (Venugopalan *et al.*, 1984). The isolated stomachs from sesbania treated rats, as well as the isolated crops of sesbania treated birds, showed paralysis. In addition, the gall bladders of sesbania treated chickens were engorged. It was unclear whether gall bladder distension was due to the inhibition of smooth musculature, an obstruction of the biliary tree or a lack of hormonal stimulus to empty since treated birds were anorexic. Obstruction of the gall bladder appeared unlikely since the activities of alkaline phosphatase and gamma glutamyl transpeptidase in serum from
treated animals were not differentially affected compared with control serum values.

The release of alkaline phosphatase and gamma glutamyl transpeptidase (GGT) is strictly governed by passage through the biliary tract. During biliary stasis and obstruction, the activity of these enzymes in the serum obtained from treated animals would be expected to rise significantly above control values. Instead, the serum activities of these enzymes were decreased compared with values obtained for sera derived from control animals. In the chicken, the liver activities of gamma glutamyl transpeptidase remained unchanged by sesbania treatment. Since the activity of this serum enzyme was depressed by sesbania treatment, there may be a problem in the release of this export protein from the liver. Enzymatic determinations of serum LDH, gamma glutamyl transpeptidase and alkaline phosphatase showed that the activities of these enzymes were all reduced in response to sesbania treatment. This suggested that liver cell damage was not the prime mechanism of action for sesbania toxins (Table 19). In addition to the decreases in chicken and rat serum GGT activities, the decreases in serum albumin concentration in both treated animal species reinforces the idea of a potential problem in export protein release from the liver as a result of sesbania intoxication.

The concentration of bilirubin in the rat, which possesses no gall bladder, was unaffected by sesbania treatment. The lack of any changes in the serum bilirubin concentrations of treated rats and decreased serum GGT and APase activities also indicated that biliary stasis was not an essential feature in sesbania poisoning.
It was of interest to determine whether the effect of sesbania extracts on AChE and PChE activities were direct. To test this hypothesis, pure enzymes were purchased and their activities assayed in the presence of sesbania extracts. The activities of the pure enzymes, true AChE derived from eel or pseudocholinesterase from horse serum, were unaffected by the addition of sesbania extracts. These results suggested that the observed decrease in PChE activities due to sesbania extracts were indirect, and therefore decreases in the serum activities of these enzymes can not be directly linked to inhibition of enzyme activity.

The observation that sesbania treated animals showed extreme abdominal vasodilatation supports the possibility of smooth muscle inhibition in sesbania intoxication. Cardiac muscle inhibition might also be a feature of sesbania intoxication. Both treated rats and chickens showed flaccid, dilated hearts and vasodilatation at necropsy. Treated animals had cold extremities, although core body temperatures remained normal. This may indicate peripheral vasoconstriction as a result of sesbania treatment. Cardiac failure, as suggested at necropsy by the presence of a flaccid dilated heart may have been responsible for the apparent vasoconstriction of the extremities. Peripheral vasoconstriction is a method for maintaining blood pressure to the vital organs of the body during cardiac insufficiency. Alternatively, the abdominal vasodilation may have been severe enough to decrease venous return to the heart, reflexively causing peripheral vasoconstriction. This aspect of sesbania poisoning needs to be further elucidated both in vitro and in vivo. A direct effect on
heart muscle in response to sesbania can not be eliminated, particularly since the heart was frequently flaccid and dilated on postmortem examination. The heart would be an obvious choice for further histochemical and electron microscopic examination of sesbania induced changes.

In the rat, the petechial hemorrhages of the lung may be related to the in vitro smooth muscle inhibition of isolated lung parenchyma reported by Venugopalan et al (1984) in chickens. These observations indicated that the lung may be an additional target organ for sesbania intoxication, an organ whose response to sesbania and its involvement in the disease process requires further investigation.

One of the characteristics of sesbania intoxication is severe weight loss. This is accompanied by a depletion of body fat stores and changes in the serum beta lipoprotein concentrations in the treated chicken but not the rat. These changes may reflect the transport of free fatty acids in the circulation as beta-lipoproteins are the normal carrier proteins for free fatty acids. In addition to changes in weight and its associated lipid stores, there was also a remarkable wasting of the skeletal muscles observed in experimentally induced sesbania poisonings. In the fowl, the pectoral muscles were frequently dark and wasted with accompanying petechial hemorrhages. This observation suggested the possibility of direct skeletal muscle involvement as a feature of sesbania intoxication. Smooth muscle inhibition by sesbania extracts has previously been noted (Venugopalan et al, 1987). Similar but less dramatic changes were noted in sesbania treated
The rats were given 1/2 the dose of treated chickens, so these species differences may have been dose related rather than true species variations. Taking into consideration the observation that sera from sesbania treated chickens or rats had, on average, about one half the protein concentration (by immunodiffusion) of the control animals, it is probable that the concentrations of alpha 2 and beta 1 fractions remained unchanged while those of albumin fell precipitously. When lipids are freed from the adipose cell, they are carried in the blood either bound to albumin or to specific lipoproteins, HDL and VLDL (Guyton, 1981). Hence the beta-1 fraction would be anticipated to increase in response to a need to have protein carriers for the free fatty acids released from adipose tissue. A specific increase in the concentration of beta-1 fraction in the chicken but not the rat (which received a lower dose) might be attributable to an increase in the concentration of circulating beta lipoproteins. The function of prealbumin is unknown. In the mouse, it is a sex dependent protein. Nevertheless, the appearance of a high proportion of this protein in the serum of sesbania treated rats indicated that there was an alteration in the synthesis and release of this export protein from the liver.

The initial observation made by Flory and Hébert (1984), that depleted circulating serum protein concentrations are a consequence of sesbania treatment, suggested the liver as a possible target organ for sesbania intoxication. Many serum proteins are synthesized in the liver. The observed decrease in circulating serum proteins could therefore be due to either an inhibition of protein synthesis or to an inhibition of export protein release from the liver.
To differentiate between these two possibilities, both serum and liver activities of representative enzymes and comparative levels of serum proteins were evaluated.

It is plausible that sesbanimide, due to its proposed similarity of action to cycloheximide (Powell et al., 1984), might decrease serum protein concentration by interfering with protein synthesis. However, differential changes in enzyme activities rather than a generalized decrease were seen in response to sesbania treatment: some glutathione-S-transferase isozymes were elevated, others remained unchanged, glutathione peroxidase was elevated while PChE, APase, LDH and PK were decreased. Protein synthesis inhibitors should non-selectively decrease the concentrations of all proteins. The rapidity of this effect depends on the protein turnover rate. There was no uniform inhibition of protein synthesis in sesbania treated animals. In liver fractions from treated animals, the activities of some glutathione-S-transferase isozymes rose significantly while others remained unchanged or decreased. Glutathione peroxidase activities, however, were lowered in the treated chicken but unchanged in the treated rat (Table 37).

The results are not consistent with the hypothesis that sesbania extracts caused inhibition of general protein synthesis. Inhibitors of protein synthesis should depress the activities of all enzymes to some degree. Differences in protein concentrations or the measured enzyme activities in response to sesbania treatment, might be explained by different protein turnover rates. If protein turnover rates were prolonged, this would account for the apparent
lack of influence of sesbania on certain enzymes. However, increased enzyme activities can only be accounted for by either an inhibition of a degradation pathway or an induction of the enzyme. It has recently been suggested that inductions of cytochrome P-450 involve both an increase in synthesis and a simultaneous decrease in the enzymes responsible for the degradation of the cytochrome (Watkins et al, 1986).

Increases in the alpha 2 macroglobulin fraction normally reflect an acute, stressful condition. It is not difficult to imagine that sesbania intoxication is an acutely stressful state. There is a marked wasting of both muscle and body fat as well as severe depletion of serum proteins. However, acute inflammation is not an accompanying feature of sesbania intoxication. In support of this is the observation that GGT activities are not elevated during sesbania treatment. It has been suggested (Singh et al, 1986) that this enzyme is a biochemical marker for inflammatory processes and that a direct relationship exists between increasing GGT activity and inflammation. Thus the acute stress of sesbania intoxication (alpha 2 macroglobulinemia) is probably not associated with inflammation. These results suggested that either sesbania toxins are small molecular weight compounds [previously noted] incapable of initiating an inflammatory response or that sesbania inhibits the body from producing an inflammatory reaction. In this regard, it is relevant to note that the gamma globulin fractions of serum were depressed in both chickens and rats undergoing sesbania treatment.
Changes were noted in the potassium balance of treated chickens. The lack of any change in the potassium concentration of treated rats may have been a function of the lower dose that rats received because of their greater susceptibility to sesbania toxins. Potassium has multifold functions in the body. These include the maintainence of acid-base balance, osmotic pressure and cell-membrane potentials (Guyton, 1981). When potassium concentrations are lowered, impaired nerve cell transmission can cause muscular paralysis. Sesbania treated birds showed muscular weakness and lethargy. These symptoms are seen with both hypokalemia and sesbania intoxication. The most important signs normally associated with changes in potassium are changes in the cardiac muscle. Decreased potassium in sesbania treated chickens may have contributed significantly to the faccid dilated hearts observed at necropsy. Changes in potassium concentration may be of importance to the overall pathology of sesbania poisoning in the chicken. It remains an area where therapeutic intervention is plausible.

Decreases in the activity of glucose-6-phosphate dehydrogenase could lead to a decrease in reducing power in the form of NADPH. NADPH is a product of glucose-6-phosphate dehydrogenase and acts as a co-factor in glutathione peroxidase activity. The decrease in the activity in the dehydrogenase may have been responsible for the slight hemolysis induced by sesbania since the peroxidase is thought to protect against membrane damage. However, the activity of glutathione peroxidase was also depressed when assayed in vitro in the presence of sufficient co-factors
Reduced glucose-6-phosphate dehydrogenase activity may also influence protein synthesis by decreasing supplies of needed RNA intermediates. This enzyme catalyzes the initial step in the pentose phosphate shunt which produces ribose and ribulose. The shunt is a reversible enzyme system which would also supply RNA intermediates from the glycolytic pathway in the form of glucose-6-phosphate which is converted to the necessary biosynthetic pentoses, ribose and ribulose. However, evidence was obtained that carbohydrate metabolism was also altered in sesbania intoxication. Pyruvate kinase activities were found to be depressed by sesbania, although chicken serum glucose concentrations were maintained during the course of sesbania treatment. The activity of hexokinase for the conversion of glucose to the phosphorylated sugar was not assessed.

Since so many of the liver enzyme profiles were found to be altered by sesbania treatment and the liver is a major detoxification organ of the body, the influence of sesbania treatment on the microsomal monooxygenase system was examined. Some conclusions as to the nature of the toxins interacting with cytochrome P-448 may be drawn. In a single experiment, sesbania was found to increase the concentration of cytochrome P-450. The activity of p-nitroanisole was unchanged by sesbania whereas resorufin ether oxidation (a specific P-448 mediated reaction) was increased by sesbania treatment in the chicken (Banton et al., 1988). A basic assumption is that the interaction of sesbania toxins with cytochrome P-448 is direct and that this interaction results in the induction of cytochrome P-448 mediated activity. The substrate
specificities of cytochrome P-448 differ substantially from the P-450 isomer (Lewis et al, 1986). Cytochrome P-450 metabolizes large, bulky more hydrophilic compounds while cytochrome P-448 interacts essentially with planar (such as sesbanimide), aromatic (not sesbanimide), highly lipophilic compounds (inconsistent with the aqueous extractability of sesbania toxins) [Lewis et al, 1986]. The distribution of the two cytochromes is genetically controlled (P-448 occurring in most tissues, P-450 being predominately confined to hepatocytes). The most specific substrate for the determination of cytochrome P-448 activity is 7-ethoxy resorufin (Lewis et al, 1986). Substrates for cytochrome P-448 are planar, rigid molecules with large size but small depth. In contrast, substrates for cytochrome P-450 are non-planar and exhibit greater flexibility of conformation. Inhibitors may act either at the active site or may bind to the heme moiety of the cytochrome. Binding to the heme portion of the molecule prevents both the binding and activation of oxygen. Thus, the substrate can not be oxygenated (Lewis et al, 1986). Many of the inhibitors which bind the heme moiety also meet the structural requirements for a substrate. This indicates that the active site is an alternate site for interaction with the cytochrome. Lewis et al (1986) have postulated, through the use of specific substrates and inhibitors that the active site of cytochrome P-448 is in close proximity to the heme moiety of this isomer.

Induction of cytochrome P-448 requires a coplanar molecule and a cytosolic binding receptor protein (Lewis et al, 1986). There is a direct relationship between the degree of binding of an inducer
to this cytosolic protein receptor and the amount of subsequent P-448 induction. Furthermore, interactions with cytochrome P-448, in contrast with cytochrome P-450 (detoxification), almost always lead to increased toxicity (metabolic activation; Lewis et al., 1986). It would be of interest to know whether the induction of cytochrome P-448 activity by sublethal doses of sesbania could lead to the promotion of tumorigenesis on subsequent exposure to a procarcinogen.

The glutathione-S-transferases are a group of isozymes that possess activity against hydrophobic, electrophilic substrates. These isozymes possess both delta^5 3-ketosteroid isomerase and glutathione peroxidase activities. These enzymes also participate in the non-covalent binding of heme and bilirubin (Yeung and Gidari, 1980). In the chicken, as in the rat, the binding of heme by cytosolic glutathione-S-transferases may modulate the intracellular concentration of the microsomal monooxygenase system. At least four soluble (cytosolic) binding proteins have been identified in the adult hen, although chickens reportedly have lower concentrations of microsomal proteins than do rats (Erlich and Larsen, 1973). Our results showed that on a milligram of protein per gram of liver basis, chickens had a higher concentration of both microsomal and cytosolic protein than did rats. On the basis of total protein, sesbania treatment increased the concentration of chicken liver cytosolic protein, whereas in rats sesbania treatment specifically increased the microsomal protein fraction. The drug metabolizing capacity of the adult hen differs from that of the rat across a spectrum of microsomal enzymes. Chickens are more enzymatically
mature than rats at two weeks of age. Induction of N-demethylation of aminopyrene increased two-fold in chickens treated with 3-methylcholanthrene compared with no induction in rats of the same age (Buynitzky et al., 1978). However, the cytosolic glutathione-S-transferase activities were reportedly equivalent (Erlich et al., 1981). Also, Erlich and Larsen (1983) reported that BNF pretreatment increased the activity of glutathione-S-transferase which remained elevated for at least 96 hours. Pretreatment of chicken with BNF increased the specific activity of the GSH-T which is important in aflatoxin metabolism. On the other hand, in the rat, BNF was less effective at inducing aniline hydroxylase activity than in the chicken (Erlich and Larsen, 1983).

In the mature chicken, sesbania and phenobarbital significantly increased GST-I microsomal activity. The microsomes from control animals represented the major site of this isozyme, although equivalent microsomal and cytosolic activities were found following sesbania treatment. In contrast, the combination treatment of phenobarbital plus sesbania caused a significant decline in microsomal activity compared with control values (Table 27). A plausible explanation for this reversal could be that the combination treatment induced GST-I activity while simultaneously increasing the degradation (turnover rate) of this isozyme in the microsomal fraction. An alternative possibility is that phenobarbital and sesbania might induce slightly different forms of GST-I and when sesbania is added following phenobarbital pretreatment, it either binds more tightly with the enzyme, making it unavailable for the subsequent metabolism of DNCB, or makes an
unstable enzyme-toxin adduct which is more readily degraded. Such a hypothesis has been proposed for some substrates of the microsomal monooxygenase system (Lewis et al., 1986) and might also hold true for GSTI.

In the cytosol of the mature chicken, sesbania induced GST-1 activity, but phenobarbital had no effect. The activity of this isozyme following the combination treatment of phenobarbital plus sesbania was significantly lower than the activity from sesbania treated animals. This suggested that there were subtle differences between the cytosolic and microsomal enzymes, with respect to their susceptibilities to induction by phenobarbital. The effect of combination treatment, as seen in microsomes, may change the turnover rate of the enzyme or may not permit the sesbania induced increases in enzyme activities or may decrease enzyme synthesis or may influence the production of functional enzyme.

This is the first report of microsomal GST-III or GST-IV in either chickens or rats. These additional enzyme activities may play a crucial role in controlling the microsomal monooxygenase system by manipulating the available heme.

The influence of age on the induction of glutathione-S-transferase activities by sesbania was determined in both immature (< 2 weeks) and mature chickens (> 6 weeks). Differences were observed between the two groups in their response to sesbania treatment. Development of the microsomal enzyme appeared to take longer than that of the cytosolic enzyme, suggesting that they were under separate genetic controls. This may also explain why the cytosolic enzyme was refractory to phenobarbital in the mature
chicken. The immature cytosolic enzymatic activity was extremely sensitive to phenobarbital induction. In the immature chicken, the combination treatment was additive. This suggested that similar forms of the enzyme were being induced by both treatments and that in the older chicken there was greater specificity of inducer.

BNF is a known inducer of cytochrome P-448 in chickens. Both sesbania and BNF treatments induced the resorufin ether activity of cytochrome P-448 (Banton et al., 1988) and GST-IV activity. GST-IV is an isozyme that is capable of metabolizing alpha beta unsaturated ketones the molecular form of one of the known conformers of sesbanimide. The induction of these activities by both sesbania and BNF may indicate a possible degradative pathway for at least one of the toxins. However, GST-IV activity was not increased in the rat and the induction of the microsomal monooxygenase system cannot be taken as direct evidence that the inducer can be a substrate for this important detoxification system.

Lipid peroxidation and the resultant membrane instability which accompanies this phenomenon are most frequently associated with the presence or production of strong electrophiles which interact with membrane lipids via a free radical mechanism. One of the prime functions of glutathione peroxidase is to maintain membrane integrity, especially by reducing lipid peroxidation. The reaction of glutathione peroxidase depends on two co-factors: NADPH supplied by glucose-6-phosphate dehydrogenase (G-6-P-DHase) and GSH. In the chicken the activity of G-6-P-DHase was depressed by sesbania treatment while GSH concentration was unaffected. Both BNF and phenobarbital pretreatments reversed the sesbania induced
decrease in GSH-peroxidase activity. However, in the rat, induction was dependent on phenobarbital pretreatment and affected the microsomal enzyme activity exclusively. The induction of this enzyme in the cytosolic fraction following sesbania treatment may reflect an induction in response to sesbania induced damage (presumably via a free radical mechanism).

Future research should be directed at determining the major enzymes involved in the detoxification of sesbania toxins. In addition to studying the interactions of purified toxins with isolated microsomal monooxygenase systems, the role of free radical generation by these toxins needs to be investigated. This will help to establish the mechanism of toxicity and the mechanism of elimination for the sesbania toxins. Research should be continued with respect to the isolation and further characterization of the toxin. Evidence has been presented in this work to establish that there is more than one toxin in crude extracts of *Sesbania drummondii*. Physical separation of the toxin responsible for weight loss from the toxin responsible for mortality, as observed in pH 2 treated extracts, could have economic potential as a possible therapeutic agent for excess obesity with a high safety index.

Studies on lung and heart as possible major target sites for sesbania intoxication need to be performed. The gross pathological findings of petechial hemorrhages (lung) and flaccid dilated hearts will require the use of histological techniques to better define the problem. The role of electrolytes in the pathophysiology of sesbania intoxication also merits investigation. Electrolyte imbalance, as an adjunct factor in accidental poisoning of cattle, needs to be furthur
elucidated. The life threatening electrolyte alterations observed in experimentally poisoned chickens may be instrumental in explaining the rapid death that is observed. Electrolyte imbalance is an area where therapeutic intervention is possible and could be a decisive factor in recovery from sesbania intoxication.

To summarize, this work supports the presence of more than one toxin in crude extracts of sesbania. This hypothesis is based primarily on the observation that the separation of different clinical signs could be achieved by manipulating physical parameters. Also, the results in the study diminish the possibility that sesbanimide is the major toxin of *Sesbania drummondii*.

This work establishes the liver as a prime target organ for sesbania induced toxicity, although other organs are also involved, especially the cardiac and respiratory systems. Sesbania toxins depress the concentration of serum proteins and selectively diminish the release of export proteins as indicated by at least one enzymatic activity. This work also describes the presence of at least two new microsomal glutathione-S-transferases which have hitherto not been identified. Initial indications suggest that one of the target sites in the liver is the microsomal monooxygenase system. These results have also indicated that there are strong species differences between chickens and rats in their responses to sesbania intoxication.
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VITAE

Marie-Lorraine Marceau-Day was born in Montreal, Canada in April of 1952. She attended St. Thomas Aquinas parochial school in Toronto until 1965 when she returned to Montreal and attended Holy Cross High School. There she was active in several extra-curricular activities including president of the Science Club, Literary Editor of the yearbook and a soloist for the Glee Club. In 1968 she received the Chemistry Prize at the Montreal Science Fair for a project entitled: The Fractional Distillation of Crude Oil. Upon graduation in 1969, she was awarded the Parent Teacher Association Scholarship Award and the Eliza M. Jones Entrance Scholarship to McGill University. As a sophomore at McGill, she was the youngest of four university students to receive and help administer an ecological research grant awarded by the Federal Government. During her tenure at McGill, she served on the student's council as a member-at-large, chairman of the student judiciary committee, carnival and wildlife committees and contributed to the school newspaper. In 1972, she won journalistic acclaim for a debate with the Quebec minister of the environment. She received the Bachelor of Science Degree in Agriculture (honors) in 1974. Following marriage and two children she moved to the University of Guelph where she spent 18 months in a graduate program in Pharmacology before following her husband, Dr. Donal F. Day to Louisiana State University. While in Louisiana, she did volunteer work at the Baton Rouge General Hospital Burn Unit. Following a few years as a research associate in the Department of Microbiology she entered graduate school in the Department of Veterinary Physiology, Pharmacology and Toxicology in the Toxicology option. In January of 1987, she had the opportunity to spend eight months in Birmingham, England doing research on Cystic Fibrosis in children. She currently has over 20 publications and presentations and is the mother of two children, Carolyn Anne, 13 and Benjamin Marc, 12. In her spare time she enjoys music, sewing swimming and bicycling.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Marie-Lorraine Marceau-Day

Major Field: Veterinary Medical Sciences

Title of Dissertation: A Study on the Toxicity of *Sesbania drummondii* in Chickens and Rats.

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination: