PARTIAL CHARACTERIZATION OF THE TOXIC EFFECTS OF *XANTHIUM STRUMARIUM* IN THE BOVINE SPECIES

By

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CHAPTER I

INTRODUCTION

Cocklebur poisoning is considered to be a major problem in swine producing areas. Swine are considered more susceptible to the toxic effects of cocklebur plants than any other species.[1,2] Cocklebur plants are more toxic to livestock when they occur in the two leaf (dicotyledon) stage. As the true leaves start to develop, the cocklebur plant becomes less toxic and is considered relatively harmless once it reaches the four leaf stage.[3,4]

Cocklebur seeds contain more of the toxic principle (carboxyatractyloside) than do the dicotyledons (two leaf stage).[4] Due to the thorny nature of the cocklebur seed, livestock losses from ingestion of the seed does not commonly occur. The greatest problem seen with the seed in livestock is the mechanical damage that occurs from the thorny spines on the seed.

The lowest lethal dose of cocklebur dicotyledons reported in swine is 1.5% of the animal's body weight.[5] Other reports consider the minimal lethal dose to be near 2% of the animal's body weight.[2] In cattle, the minimal lethal dose is reported to be 1.8% of the animal's body weight.[2] Sheep must consume higher amounts of cocklebur dicotyledons than pigs or cattle before death occurs.[2,5] Cocklebur seeds must be fed at 20 to 30% of a swine ration in order to cause lethal effects.

Carboxyatractyloside is considered to be the toxic compound found in Xanthium strumarium (cocklebur).[3,4] Early reports suggested that xanthostrumarium (a glycoside) was the toxic agent in cocklebur while later reports stated that hydroquinone was the cause of the signs of toxicity seen in cocklebur poisoning.

Carboxyatractyloside is a diterpenoid glycoside that is also a potential uncoupler of oxidative phosphorylation. It has been proven experimentally to cause hypoglycemia in laboratory animals as well as in swine.[6]

Hypoglycemia (abnormally low blood glucose levels) is a condition that most commonly occurs in neonates less than 4 weeks of age. This condition in neonates is usually caused by lack of appetite which may be secondary to disease, reduced availability of milk (underfeeding or poor production by dam), or feeding with a poor quality or improperly prepared milk replacer. Low environmental temperatures may also compound the problem of hypoglycemia due to higher energy requirements for thermoregulation.[7,8]

Hypoglycemia in older animals is less common and the etiologies more varied. In older animals common causes of hypoglycemia are toxic and neoplastic insults to different target organs. The most important of these organs are the pancreas and the liver since these are the major organs that control the blood glucose levels in livestock.

When the blood glucose levels are low, the pancreas produces the hormone glucagon. Glucagon stimulates gluconeogenesis and glycogenolysis thus elevating glucose levels. When blood glucose levels are elevated, the pancreas produces insulin which decreases glycogenolysis and increases cellular uptake of glucose.[9] The liver is the storage

organ for glycogen (the stored form of glucose) and responds to the insulin and glucagon levels in the blood. Insulin decreases glycogenolysis (breakdown of glycogen to glucose) and glucagon increases glycogenolysis in the liver. The liver in ruminants also converts volatile fatty acids to glucose.

Recently, there has been an increased number of cases sent to the Oklahoma Animal Disease Diagnostic Laboratory of suspected cocklebur poisoning in cattle. The only lesions seen were gross and histopathologic liver damage. The cases occurred mainly in the late spring during the time in which cocklebur seeds germinate. Further investigation proved that the cattle were grazing pastures that contained considerable amounts of cocklebur plants. It was determined from clinical signs, histopathologic lesions, and evidence of grazing the cocklebur plants in the dicotyledonary stage that the probable cause of death was cocklebur poisoning.[10]

Due to the increased number of suspected cases of cocklebur poisoning in cattle, the following study was undertaken to:

1. Determine the clinical signs in calves experimentally poisoned with cockleburs.

2. Partially characterize the clinical pathology and histopathology seen in calves exposed to cocklebur plants.

3. Attempt to determine if the cause of death is due to the hypoglycemic effects of carboxyatractyloside (CAT) from Xanthium strumarium (cocklebur).

CHAPTER II

LITERATURE REVIEW

Previous Work with Cocklebur

Xanthium species was described as a poisonous plant to livestock in 1880 by Dr. Joseph Bancroft in a paper presented to the Queensland Physiological Society. This paper reported a large number of cows, one horse, and a sheep had been killed on a single private estate in Australia by eating young plants of Xanthium strumarium. The animals reportedly fell upon the ground and died very quickly. Bancroft obtained the same results by orally poisoning small animals with an extract from the plant Xanthium spinosum.[5]

In 1881, the name Xanthostrumarium was given to the toxic fraction with glycosidic characteristics isolated from cocklebur seeds. Cheatham (1884) stated that cocklebur in the United States were one of the first plants that appeared in the spring and that in some of the southern and western states swine that were allowed to graze would invariably die after eating the plant.

Many of the early reports of toxicity and death due to species of Xanthium was attributed to mechanical injury caused by the spiny bur and not a toxin from the plant itself (1898-1920).[1,5]

Early experimental work done with Xanthium spp. (1920-1923) was done at Salina Experiment Station and at the Bureau of Animal Industry

Experiment Station, Bethesda, Maryland. There were, in all, 67 experiments with swine, 11 with sheep, 12 with cattle, and 19 with chickens.

Postmortem examinations were performed on 7 pigs, 1 sheep, 2 cows, and 2 chickens. The outstanding features in all species were the lesions in the alimentary canal, the liver, and to a lesser degree the glandular structures. In the pigs, the stomachs were congested, while in cattle congestion was present in the abomasum and to a lesser degree the duodenum, ileum, jejunum, and colon. In sheep and pigs, the liver was congested and spotted; while in cattle it was bluish. In all species walls of the gall bladders and occasionally the common bile ducts were thickened and the bile was thick and viscid. In some cases, there was an accumulation of a serous infiltrate surrounding the gall bladder and bile ducts and excess serosanguineous fluid in the abdominal cavity. These conditions varied in many animals and were not uniform. The only uniform finding in the pig was the congested stomach.[5]

Many other reported cases of poisoning by Xanthium spp. have occurred since that time and there are almost as many reports in the literature that Xanthium is completely innocuous.[11,12] In 1949, Kutzel and Miller determined that Xanthium was toxic to animals and the toxicity was due to a quinine-type structure, hydroquinone.

Kutzel and Miller did a comparative chemical study of the bur and the kernel. The plant material was obtained from several sources in North Dakota. The burs were identified as belonging mainly to the species Xanthium canadense. A hydroalcoholic extract was prepared and processed for alkaloids with negative results. A 10% aqueous extract was prepared from the kernels and tested for toxicity in mice. The extract was found to be toxic. Reduction tests were done on the extract,

Folin-McElroy reagent, Benedict's and Fehling's solutions. As a result of the reduction tests, an attempt was made to isolate the agent responsible for the reactions.

The Bay-Gisvold method of extraction was used on the kernels.[13] A yellow crystalline substance was obtained. The substance was tested for toxicity and proved to be quite toxic to experimental animals causing identical clinical signs as those seen in field cases of cocklebur poisoning. The solvents furon and tetrahydrofuron were found to produce the most pure crystalline deposits.

The crystalline material was characterized and found to have a melting point between 168° and 169°C. The crystals were soluble in ether, ethanol, and benzene. The material was soluble in cold water at 5 parts per hundred, very soluble in hot water, and insoluble in chloroform. With sulfuric acid, a rust-brown color was formed. With nitric acid, a bright green color was produced, along with nitrogen oxide gases.

Sodium fusion was done to determine if nitrogen, sulfur, phosphorus, or halogens were present. None were found. Reduction tests were done on copper solutions and were all positive. Attempts to form osazones, 2-4 dinitrophenyl hydrozones were unsuccessful. The material was optically inactive. The pH of a 1% solution was 6.88. There was no reaction with Schiff's reagent and the Folin-Denis test for free phenolic groups was positive. From this data a quinone-type structure was suspected. It was observed that hydroquinone (Figure 1) possessed the same physical properties as the unknown substance and by experimentation, hydroquinone was found to possess the same chemical properties as the newly isolated substance. Spectrophotometrically the unknown crystalline material and

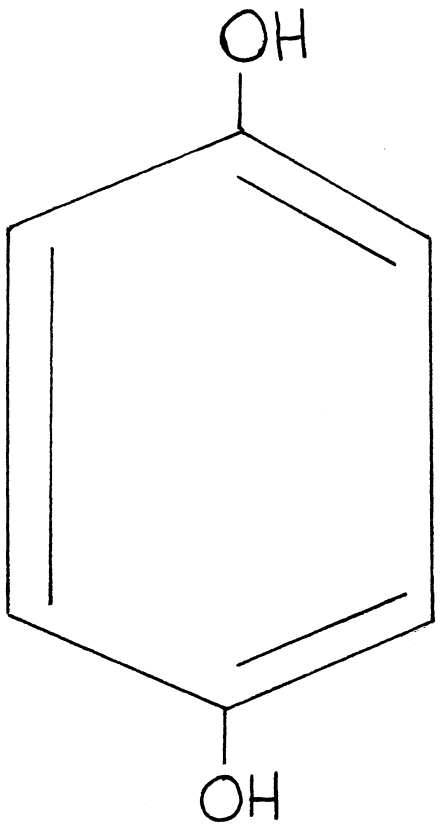

Figure 1. Hydroquinone

the standard of hydroquinone matched. Pharmacological experiments in mice showed that the toxic symptoms seen with Xanthium poisoning were reproducible with the crystalline isolate and pure hydroquinone. Necropsy findings revealed small, individual differences but in general the changes were very similar. There were subcutaneous hemorrhages, localized hemorrhage of the liver, spleen, and kidney and heavy, serous infiltrate in all body cavities.[11]

In 1978, Cole et al. began to re-examine the etiologic agent in cocklebur as a result of an outbreak of cocklebur poisoning in south Georgia. They were not able to detect hydroquinone in cocklebur nor able to reproduce with hydroquinone the characteristic lesions associated with cocklebur toxicity.

Burs of Xanthium spp. were collected in fields of southern Georgia. Some burs were ground in a Wiley mill and some were germinated. The two leaf stage (seedlings) and older seedlings (five leaf stage) were collected, lyophilized, and ground in a Wiley mill.

The toxicity of the crude extracts and other preparations was monitored with 30 to 40 lb shoats. The initial crude preparations were given orally and purified toxin was administered intravenously in sterile distilled water.

A semiquantitative isolation was done on the burs to identify the toxin carboxyatractyloside (Figure 2). Thin layer procedures were used to compare hydroquinone to carboxyatractyloside. All direct analytical comparisons of the toxin from cocklebur with an authentic sample of potassium carboxyatractyloside confirmed the identity of the toxin as carboxyatractyloside. The amount of carboxyatractyloside was 0.457% for

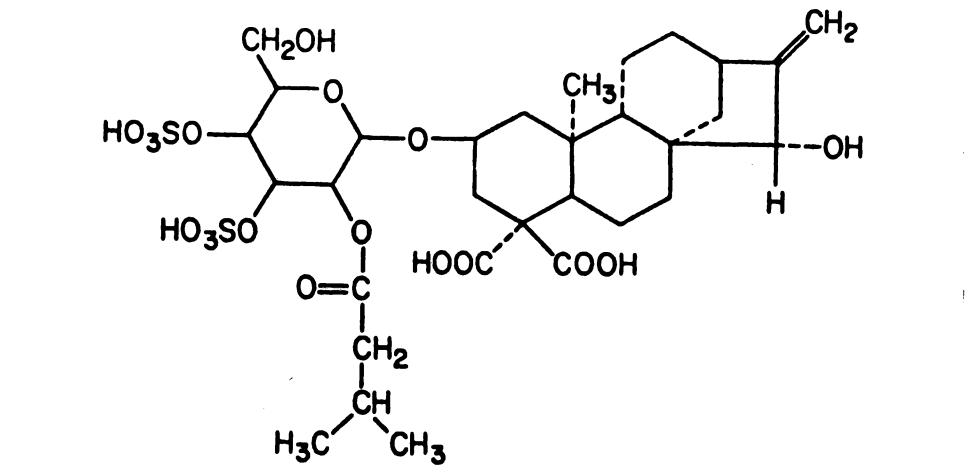

Figure 2. Carboxyatractyloside

burs and 0.12% for seedlings (two leaf stage). Carboxyatractyloside was not detected in the older seedlings (four leaf stage).[4]

Daniel et al. (1972) first isolated the glycoside carboxyatractyloside from the rhizomes of Atractylis gummifera. Then, in 1974, Kupiecki et al. isolated a hypoglycemic agent from cocklebur (Xanthium strumarium) that was later identified as carboxyatractyloside by Craig et al. (1976).[14] The hypoglycemic effect was not associated with the specific diagnostic changes seen in swine caused by cocklebur poisoning. Cole et al. did further work with the previously reported toxic principle hydroquinone. They were unable to detect any hydroquinone in X. strumarium nor were they able to reproduce the typical lesions of Xanthium toxicity after oral administration of authentic hydroquinone at levels up to twice the lethal dose reported by Kutzel and Miller (1950). When Cole et al. used purified carboxyatractyloside the clinical signs and typical lesions (ascites and hepatic lesions) were indistinguishable from the signs and lesions seen with cocklebur seedlings.

Dose of Plant to Cause Toxicity

The amount of cocklebur plant (seedling, two leaf stage) necessary to cause clinical illness and death varies among reports found in the literature.[2,3,5] There is also a reported species variance associated with cocklebur poisoning.[2] In swine, the smallest toxic dose (dose that would produce clinical signs) reported by Marsh et al. was 0.736% of the body weight of pigs. The minimal lethal dose (smallest lethal dose) reported by Marsh et al. (1924) was 1.496% of the pigs body weight. Marsh et al. also did experiments with sheep and cattle and found a dose of 1.543 pounds of cocklebur dicotyledons per hundred weight killed one

sheep and 2.911 pounds of dicotyledons per hundred weight killed cattle. Marsh also found that lethal effects occurred in chickens given doses at 6% of their body weight in cocklebur dicots.

Research done by Kenny et al. (1950) with Xanthium pungens (Noogoora bur) showed that the lethal dose of cocklebur dicots in pigs and calves was about 2% of the pigs' body weight and 1.8% of the calves' body weight. They determined that sheep needed to consume a considerable amount of their body weight (much higher than calves and pigs) to cause death.

Stuart et al. (1981) did further work in swine with cocklebur dicots and seeds. They found that the minimal lethal dose in swine was 0.75% of the animal's body weight in cocklebur dicots. They increased the dose of cocklebur dicots to 3% of the pigs' body weight and had 4 out of 4 animals die. At 1.5 and 2.0% of the animal's body weight, 2 out of 4 pigs died in each group. There were no clinical signs seen in pigs fed 5% of their body weight in a divided dose over a period of 14 days. This corresponds with Marsh et al.'s findings that the toxic principle was not cumulative. The dose of ground bur necessary to cause death in swine was found to be around 20% of the ration fed.

Pigs fed older plants (four to 6 leaves) at 3% of their body weight did not show any clinical signs after 7 days. In general, the toxic dose of cocklebur dicots for pigs has been estimated around 2% of the body weight; in cattle, 1.8%; and in sheep, higher levels than in pigs or cattle.[2,3]

Clinical Signs of Cocklebur Poisoning

The clinical signs associated with cocklebur poisoning in swine are

characterized by depression, tucked appearance, occasional nausea, incoordination, spasmodic movement, lateral recumbency, convulsive paddling, and coma. Death usually occurs within 48 hours and often within 3 to 4 hours following ingestion of the cocklebur dicots.[1,2,3]

The clinical signs seen in other species are similar to those seen in swine. In cattle, signs that have been observed are weakness, depression, belligerence, recumbency, and convulsions, with death following in most cases.[5,10,15]

Gross Lesions of Cocklebur Poisoning

The principle gross lesions seen in pigs associated with carboxyatractyloside toxicity are related to increased vascular permeability characterized by serofibrinous effusions in the peritoneal cavity and to a lesser extent in the thoracic cavity, pericardial sac, and subcutaneous tissues. Marked edema is present in the gall bladder wall, gastrohepatic ligament, and periductular tissue surrounding the bile duct.

The gastrointestinal lesions seen range from mild congestion of the stomach mucosa to extensive gastroenteritis.[2,3,5,16]

In cattle, the principle gross lesions are spotted, congested liver, serous thickening of the gall bladder wall and common bile duct, splenic and abomasal congestion.[5,10] Initially, the thicker gall bladder wall was believed to be due in part to flukes with which the cattle were infested.[5]

Biochemical Mechanism of Toxic Principle

Carboxyatractyloside

In 1971, Inverni and Della Beffa found that carboxyatractyloside, a

structural derivative of atractyloside was an active inhibitor of adenine nucleotide translocation. The amount of carboxyatractyloside needed to inhibit coupled respiration is about ten times less than that of atractyloside. Carboxyatractyloside becomes firmly bound to the mitochondrial membrane thus preventing phosphate uptake. The prevention of phosphate uptake is possibly due to the allosteric effects of carboxyatractyloside. These effects are similar to those proposed for atractyloside by Vignais in 1966.[17]

Hypoglycemic Effect

In 1974, Kupiecki et al. isolated and characterized a hypoglycemic agent from Xanthium strumarium. They compared the hypoglycemic activity of cockleburs in rats by taking crude extracts and partially purified crystalline plant product and variably dosing the rats. The results showed a good dose-response relationship for each step of purification. Blood glucose levels were not affected 1 hour after dosing. After 2 hours, glucose levels were obviously lowered, but maximum decrease occurred by 3 hours and persisted at that low level through hours 5 and 7. Kupiecki et al. also tried to eliminate the possibility that the drug acted directly on the liver when injected into the peritoneal cavity. They administered the drug subcutaneously and produced equal effects on blood glucose level. Orally, the crude material, purified extracts, and crystalline compound had weak hypoglycemic activity even though it was given at 3-5 times the injected doses. After partial characterization of the compound, it was concluded that the agent responsible for the hypoglycemic effect was not any of the previously isolated compounds (xanthostrumarin, hydroquinone).[6]

Later, Craig et al. isolated and identified the hypoglycemic agent from Xanthium strumarium. The whole, ground burs were extracted with different solvents. The residue acquired after these procedures was determined to be an insoluble glycoside. The glycoside was purified by forming the potassium salt and then recrystallized. Direct comparison of the potassium salt of the glycoside with an authentic sample of potassium carboxyatractyloside confirmed the identity of the isolated glycoside as carboxyatractyloside.[14]

Isolation and Redefinition of Toxic Principle

In 1980, Cole et al. isolated and redefined the toxic agent in Xanthium strumarium (cocklebur). They determined that the highly toxic agent responsible for the poisonous properties of cocklebur was carboxyatractyloside. The toxin was identified by spectroscopic and chemical comparisons with authentic carboxyatractyloside.

Further work was done by Stuart et al. in 1980 with cocklebur plants and ground burs. The toxicity of the plant in different stages of development as well as the burs was assessed. The lesions seen when pigs were intoxicated with plants and burs were compared to lesions seen with authentic carboxyatractyloside and hydroquinone. This experiment supported earlier work that carboxyatractyloside was the toxic agent found in cocklebur.[4,6,18]

Description of Cocklebur Plant

Cockleburs (Xanthium spp.) are coarse annual herbaceous weeds that grow all over the world. They occur in fields and waste lands, but especially in areas where receding water has exposed previously

submerged land, as along the shores of ponds or rivers and in flood plains.[1]

The most consistent differences in local populations of plants have been found in the structure of the bur. Some botanical taxonomists separate the cockleburs into a score of species on the basis of these differences. If differences in bur structure are not used to separate species, only two or three species are recognized. The most common species being Xanthium strumarium, L. var. strumarium.

Xanthium strumarium is a coarse annual herbaceous weed which may reach 3 ft or more in height. The stems are erect, branched, and stout. Leaves alternate, petiolate, deltoidovate, more or less cordate, rough-surfaced, and blades may be up to 0.5 ft long and almost as wide. The margins of the leaves are irregularly cut, toothed, lobed or almost entire. In florescence, plants have a modified composite head; with sexes separated. Male heads are small and are grouped in terminal or axillary racemes. Female heads are composed of two florets which lack rays and are surrounded by a many-bracted, elongate involucre. This entire structure becomes the fruit. The tips of the involucral bracts turn outward and become more or less hooked with woolly spines. This bur structure is characteristic of the genus.[1]

Xanthium spinosum L. is quite different in appearance. It is smaller; bears lanceolate, trilobate leaves; and forms a conspicuous, 3-pronged spine in each leaf angle.

Both of these species are found throughout the United States and Canada. Xanthium pungens (Noogoora bur), which is very similar to Xanthium strumarium, is found in Australia.[12]

Causes of Hypoglycemia

Hypoglycemia is a decrease in blood sugar that most commonly occurs in neonates less than four weeks old. Kirk has divided the causes of hypoglycemia into two categories. Group one includes functional tumors of pancreatic islet cells, excess exogenous insulin, or by feeding leucine or sulfonylurea. Other conditions in group one include severe muscular exercise which causes increased utilization of glucose and congenital renal tubular defects which can result in excessive removal of glucose from the body. Phlorhizin (a nephrotoxin) can cause similar effects as congenital renal tubular defects. Group two includes inadequate blood glucose levels due to starvation, malnutrition, malabsorption, severe liver damage due to poisons or disease, or deficient glycogen storage.[19]

Hypoglycemia can be due to excess circulating insulin which causes a decrease in glycogenolysis and an increase in cellular absorption of glucose by fat, muscle, and liver cells.

The excess in insulin could be due to iatrogenic treatment of animals (dogs) for diabetes mellitus. Insulin levels can become excessive from secretion by functional beta cell tumors of the pancreas. Supplementing diets with amino acids like leucine can cause hypoglycemia through excessive feeding. Leucine is an amino acid that is ketogenic. High levels of leucine will produce ketone bodies in ruminant species. Ruminants use oxalacetate to break down ketones as well as produce glucose. Once the levels of ketones become excessively elevated, the oxalacetate is depleted thus reducing glucose production.[7,19]

Phlorhizin comes from the root bark and bark of apple, pear, plum, and cherry trees. It is also found in the leaf and leaf buds of apple trees. The compound has been recommended as an additive for lubricating motor oils and causes glycosuria experimentally in animals. It is believed to cause renal tubular defects allowing excessive glucose to be released into the urine.

Clinical Signs of Hypoglycemia

The clinical signs of hypoglycemia may vary proportionally with the severity of the hypoglycemic condition. Severe depression, coma, and on rare occasions seizures are seen. Because the brain is highly dependent on glucose for energy, normal function can not continue in a hypoglycemic state. Also, bradycardia may be present in primary hypoglycemia.

Lesions of Hypoglycemia

Hypoglycemia, which causes a disturbance of intracellular respiration, is usually classified with cellular anoxia. Oxygen is available to nervous tissue but cannot be utilized due to a reduction in the amount of substrate needed for cellular respiration. In spite of the severe convulsions and deep coma that occur during hypoglycemic episodes, there are no conspicuous changes in the brain. There is probably a biochemical disturbance in the neurons that precedes histologic signs of degeneration by a considerable period. Death may occur too rapidly for recognizable lesions to develop. When coma is prolonged, there are changes in neurons but the hypoglycemic changes cannot be readily differentiated with light microscopy from autolytic and nonspecific changes. When there are neuronal changes present, they include

chromatolysis, disappearance of the cytoplasmic margins and fading of the cytoplasm, pyknosis, nuclear eccentricity, and fading of the nucleus.[16]

CHAPTER III

MATERIALS AND METHODS

Cocklebur Dicotyledons

Burs from the adult plant Xanthium strumarium were collected from various locations in Payne County, Oklahoma, for germination purposes. The burs were planted and germinated in 3 in. by 20 in. by 9 in. trays and allowed to grow until they reached the two leaf-dicotyledonary stage. At this time, the dicotyledons were harvested at surface level and stored frozen until enough plant material was collected to administer to calves. The plants were weighed and made into a slurry by blending in a Waring commercial blender. Sufficient distilled water was added to the blended plants until the consistency of the material was such that it could be pumped by stomach tube into calves.

Representative samples were taken from cocklebur dicotyledons collected for each phase of the experiment to determine moisture content. One-hundred gms of wet weight cocklebur dicotyledons were taken from the plants collected in each phase. The plants were oven dried for approximately 12 hours at 75°C and weighed to determine dry weight.

Phase I: Determination of Lethal Dose

Five newly weaned calves (approximately 2 months of age) were acquired from Braum's Dairy in Tuttle, Oklahoma. The calves were kept

in open stalls and allowed free access to alfalfa hay and water as well as a ration containing oats, alfalfa pellets, and corn. All calves were weighed and initial blood and serum chemistry values were determined for each calf to determine their general state of clinical health. The calves were allowed to acclimate for a one week period before they were dosed with the slurry made from cocklebur dicotyledons.

Lethal Dose

The LD50 dose from the literature for cocklebur dicotyledons in cattle (1.8% body weight of wet weight dicots) was used as a reference point for dosing the calves in this phase of the experiment. The calves were identified as calf #1, calf #2, calf #3, calf #4, and calf #5.

Calf #1 was given 2.0% of its body weight in wet weight cocklebur dicotyledons; calf #2 was given 1.0%; calf #3 was given 0.5%, and calf #4 was given 0.75%.

Calf #5 was given 50.0 mg of dipotassium sodium carboxyatractyloside from the thistle Atractylis gummifera.[a] The carboxyatractyloside (CAT) was dissolved in 30.0 ml of physiologic saline and given to calf #5 by slow intravenous injection (jugular). Pure CAT was given to assure that the cause of the signs and lesions seen were due to CAT in cocklebur dicotyledons.

Blood Collection

Blood samples were obtained by jugular venipuncture on all calves at the time of dosing (zero hour). The blood was placed in EDTA tubes

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[a]Sigma Chemical Company, P.O. Box 14508, St. Louis, Missouri.

for hematologic examination and clot tubes for serum chemistries. Blood was also taken in potassium oxalate/sodium fluoride tubes for glucose levels.[b] The blood from the clot tubes was spun down in a Dynac centrifuge[c] within 10 minutes after collecting the samples. The blood samples were then taken to the Clinical Pathology Laboratory, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma, for analysis. White blood cell (WBC), hemoglobin (Hb), hematocrit (Hct), total protein (TP), glucose, and differential white blood cell counts were done on the whole blood.

Blood urea nitrogen (BUN), creatinine phosphokinase (CPK), sorbitol dehydrogenase (SDH), and serum gamma glutamyl transpeptidase (SGGT) were the serum chemistries that were analyzed.[d]

The serum electrolytes calcium (Ca), chloride (Cl), magnesium (Mg), sodium (Na), and potassium (K) were determined in calf #1. Samples for the above analyses were taken at 0, 1, 5, and 11 hours following administration of cocklebur dicotyledons.

Clinical signs were monitored when blood samples were taken from each calf.

Gross Pathology

Systematic necropsies were done on each calf that died or following euthanasia with intravenous sodium pentobarbital at the end of post-inoculation day 7.

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[b]Vacutainer®, Vacutainer Systems, Rutherford, New Jersey.

[c]Dynac centrifuge, Clay Adams Division of Becton Dickerson and Company, Parsippany, New Jersey 07054.

[d]Dupont ACA® Automatic Discrete Chemical Analyzer, E.I. Dupont de Nemours and Company and Instrument Systems, Wilmington, Delaware.

The following tissues were collected from each calf in Phase I: brain, lung, left ventricular papillary muscle, liver, kidney, abomasum, rumen, reticulum, omasum, small and large intestine, pituitary, gall bladder, lymph nodes, and pancreas. All tissues were fixed in buffered 10% formalin except the brains which were fixed in 30% formalin.

Histopathology

Sections for histopathologic examination were embedded in paraffin (processed in a Lipshaw Trimatic automatic tissue processor[e]), cut 4 to 6 micrometers thick, and stained with hematoxylin and eosin in a Fisher automatic stainer[f].

Partial Characterization of the Lethal Effects of Cocklebur Dicotyledons in Calves (Phase II)

In Phase II four weaned calves acquired from Braum's Dairy were used. The calves were identified as calf #6, calf #7, calf #8, and calf #9. Each calf was acclimated as in Phase I. Each calf was given a lethal dose of cocklebur dicotyledons as determined in Phase I. In Phase I, four calves were dosed with cocklebur dicotyledons. Calf #1 was given 2% of its body weight in cocklebur dicots, calf #2 was given 1% of its body weight in cocklebur dicots, #3 was given 0.5% of its body weight in cocklebur dicots, and #4 was given 0.75% of its body weight in cocklebur dicots. It was determined since only calves #1 and #2 died

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[e]Lipshaw Trimatic, Lipshaw Manufacturing Corporation, Detroit, Michigan.

[f]Fisher automatic stainer, Fisher Scientific, Pittsburgh, Pennsylvania.

the minimal lethal dose was 1% of a calf's body weight. The 1% level of cocklebur dicots was used in Phase II.

Blood Collection

Blood samples were obtained by jugular venipuncture as in Phase I. The blood was collected at the time of administering cocklebur dicotyledons (zero hour), 5 hours post-administration, and finally every 2 hours until death. Blood was collected in Ca EDTA tubes, potassium oxalate/sodium fluoride tubes, and clot tubes.

Clinical Pathology

In Phase II the clinical pathologic analyses done were reduced to RBC, WBC and different counts, SGGT, BUN, and glucose levels. The number of tests performed were reduced because of cost and values seen in Phase I.

Insulin Levels

Blood was collected in clot tubes and centrifuged to acquire serum for insulin analysis. Serum was sent to the Texas A&M Diagnostic Laboratory for insulin analysis by rapid solid-phase radioimmunoassay. Due to economic factors, serum samples were only sent from three calves (#6, #7, and #9). The samples were taken at zero hour following administering cocklebur dicotyledons and a sample was taken as close to death as possible.

Gross pathologic and histopathologic examinations were performed in the same manner as in Phase I.

CHAPTER IV

RESULTS

Wet Weight vs Dry Weight of Cocklebur Dicotyledons

One hundred grams of cocklebur dicotyledons were taken from representative samples of dicotyledons used to dose calves in Phase I and Phase II to determine moisture content. The samples were oven dried and then weighed after complete dryness. Cocklebur samples used in Phase I contained 89% moisture (dry weight 11 gm) and cocklebur samples in Phase II contained 91.4% moisture (dry weight 8.6 gm).

Lethal Dose of Cocklebur (Phase I)

Calf #1, when administered orally 2% of its body weight in cocklebur dicotyledons, died within 11 hours. Calf #2 was given 1% of its body weight in cocklebur dicotyledons and died within 22 hours. Calves #3 and 4 were given 0.5% and 0.75% of their body weights in cocklebur dicotyledons and were euthanatized 1 week postinoculation after exhibiting no clinical effects. Based on the results from these 4 calves, 1% of the calf's body weight in cocklebur dicotyledons was considered the minimum lethal dose and was used for Phase II of the experiment.

Clinical Observations

Clinical observations will be discussed separately for each phase of the experiment. Calves #1, 2, 3, 4, and 5 were included in Phase I. Calves #6, 7, 8, and 9 were in Phase II.

Phase I

The amount of cocklebur dicotyledons received by each calf per kilogram body weight is presented in Table I. Table II lists a summary of clinical signs seen in Phase I.

Calf #1 received 2% of its body weight in cocklebur dicotyledons. For 10 hours after dosing by stomach tube, calf #1 remained clinically normal. After 10 hours the calf was found in sternal recumbency with its hindlimbs splayed behind it. The calf was weak and depressed but showed no evidence of struggle. The calf died 30 minutes after being found in sternal recumbency.

Calf #2 was given 1% of its body weight in cocklebur dicotyledons. The calf remained clinically normal for 20 hours after dosing. The calf was then found 21 hours after dosing in sternal recumbency with its hindlimbs splayed behind it and exhibiting excessive muscle tremors. Twenty minutes later, the calf started paddling convulsions and was hypersensitive to sound. The calf frequently exhibited opisthotonos following intermittent convulsive seizures. The calf died 20 minutes after the seizures began.

Calves #3 and #4 were given 0.5% and 0.75% of their body weight in cocklebur dicotyledons. These two calves showed no clinical signs of

TABLE I

AMOUNT OF COCKLEBUR DICOTS RECEIVED BY EACH CALF PER KG BODY WEIGHT

Phase	Animal	% Body Weight Dicots[a]	Body Weight (kg)[b]	Weight of Dicots (kg)[c]
1	1	2	78.0	1.560
	2	1	90.0	0.900
	3	0.5	71.4	0.360
	4	0.75	87.3	0.650
	5	received 50 mg CAT	115.5	---
2	6	1	85.5	0.855
	7	1	84.6	0.850
	8	1	74.6	0.745
	9	1	74.6	0.745

[a]Percent body weight of dicotyledons given to each calf.
[b]Calf weight (kg).
[c]Dicotyledon weight (wet weight) in kilograms.

TABLE II

CLINICAL SIGNS OF CALVES TREATED WITH DIFFERENT DOSES OF COCKLEBUR DICOTYLEDONS

Calf No.	Dose of Dicots	Time of Onset of Clinical Signs	Clinical Signs Observed
1	2%*	10 hours	sternal recumbency, hindlimbs splayed behind, weak, depressed. Calf died on 11th hour postinoculation.
2	1%	21 hours	sternal recumbency, splayed hindlimbs, muscle tremors, lateral recumbency 20 minutes later, paddling convulsions, hypersensitivity to sound and touch, opisthotonos. Died 20 minutes after seizures.
3	0.5%	---	No clinical illness seen.
4	0.75%	---	No clinical illness seen.
5	50 mg CAT	---	Normal at 8 hours postdosing. Found dead 11 hours after dosing.

*Percent body weight given of dicotyledons (wet weight).

illness for a week after dosing. At the end of the one week period, the calves were still clinically healthy and were euthanatized.

Calf #5 was given 50 mg of purified carboxyatractyloside (CAT) by slow intravenous injection. Eight hours after dosing the calf was clinically normal. Eleven hours after dosing the calf was found dead in lateral recumbency with rigor mortis already present.

Phase II

Table III is a summary of the lethal effects of cocklebur dicotyledons given at 1% of the calves' body weight.

Calf #6 was clinically normal until 16 hours after dosing when the calf was found in sternal recumbency. The calf was slightly depressed and would not rise when stimulated, but continued to ruminate and chew its cud. At 18 hours post-dosing, the calf was in lateral recumbency and nonresponsive to sound or touch. The calf's pulse, temperature, and respiration varied little until just before death.

Calf #7 was clinically normal after 14 hours post-dosing. During the 16th hour, the calf developed sternal recumbency and became depressed. Eighteen hours after dosing the calf was laterally recumbent and convulsing with violent paddling motions. The paddling kicking motions of the hindlimbs would almost touch the sternum. The calf died 30 minutes after the convulsive seizures began.

Calf #8 did not show any signs of illness until 25 hours after dosing. At that time the calf was in sternal recumbency and would not get up. The calf got progressively worse over the next 4 hours. By 30 hours postdosing, the calf showed muscle fasciculations and was so depressed that when the animal's head was placed in the flank area to

TABLE III

LETHAL EFFECTS OF COCKLEBUR DICOTYLEDONS GIVEN AT 1% OF CALVES' BODY WEIGHT

Calf No.	Time of Onset of Clinical Signs After Dosing (hrs)	Clinical Signs Observed
6	16 hours	sternal recumbency, depressed 2 hours later, lateral recumbency, nonresponsive to sound or touch, died 5 minutes later
7	16 hours	sternal recumbency, depressed 2 hours later, lateral recumbency, convulsions, violent paddling motions, died 30 minutes later
8	25 hours	sternal recumbency, depression, would not rise upon stimulation, muscle fasciculations, ataxia (over next 24 hrs improved), found dead 72 hours after innoculation
9	14 hours	sternal recumbency, depression, when laterally recumbent extensor rigidity, hypersensitive to touch and sound, convulsions (paddling) when stimulated, died 5 minutes after convulsing

draw blood the calf would not move it from that position for 5 minutes or more. Over the course of the next 24 hours the calf seemed to improve. Sixty hours postdosing, the calf would rise but was ataxic. Seventy-two hours after dosing the calf was found dead already in a state of rigor mortis.

Calf #9 was clinically normal until 14 hours post-dosing. At that time the calf was in sternal recumbency and was very depressed. If the calf was forced into lateral recumbency, it would go into extensor rigidity and would be hypersensitive to touch. At 15 hours post-dosing the calf was in lateral recumbency and had muscle fasciculations. If touched or stimulated with loud noises, the calf would start a paddling convulsive type seizure. The calf died about 5 minutes after developing seizures.

Clinicopathologic Observations

Hematologic Examination

Blood obtained before the calves were dosed was used as normal baseline hematologic values (see Tables IV and V). Blood was taken as close as possible to the time of death or euthanasia to compare pre-dosing hematologic values to postdosing values. Blood was not taken from calves #4 and 5 before the time of death or euthanasia. Calf #8 lived 3 days after dosing with dicotyledons and blood was taken on day 2.

Clinical Chemistry

Extensive clinical chemistries were only taken for calf #1. Values are presented in Table VI. From the chemistry values determined from

TABLE IV

EFFECTS OF VARIOUS LEVELS OF COCKLEBUR DICOTYLEDONS ON HEMATOLOGIC VALUES

Calf #	Dose of Cocklebur % B.W.	WBC ($\times 10^3$)		Hb (g/dl)		HCT (%)		TP (g%)	
		Before	After	Before	After	Before	After	Before	After
1	2	6.5	24.7	11.5	11.6	36.0	34.5	6.8	5.9
2	1	16.5	42.6	11.9	13.0	39.0	43.0	7.0	6.2
3	0.5	13.2	10.8	10.9	12.2	35.5	37.5	6.6	6.7
4	0.75	8.9	--	10.3	--	33.5	--	7.2	-
5	50 mg CAT	11.6	--	11.4	--	36.5	--	8.5	-
$\bar{x} \pm$ SE		11.3±1.72	26.03±9.2	11.2±0.27	12.26±0.4	36.1±0.88	38.3±2.48	7.22±0.33	6.26±0.23

- = Values were not taken.
WBC = white blood cell; Hb = hemoglobin; HCT = hematocrit; TP = total protein.

TABLE V

THE EFFECTS OF LETHAL DOSES OF COCKLEBUR ON HEMATOLOGIC VALUES

Calf #	Dose of Cocklebur Received*	WBC ($\times 10^3$)		Hb (g/dl)		HCT (%)		TP (g%)	
		Before	After	Before	After	Before	After	Before	After
6	1	12.5	17.2	12.6	12.1	40.5	37.5	7.1	7.0
7	1	7.7	21.1	9.8	10.9	30.0	34.0	7.1	7.6
8	1	17.4	13.7	10.6	10.4	34.0	34.5	7.9	7.4
9	1	12.3	23.0	11.8	13.7	36.0	39.0	7.2	7.1
$\bar{x} \pm$ SE		12.47±1.98	18.75±2.07	11.2±0.62	11.76±0.73	35.1±2.18	36.2±1.19	7.32±0.19	7.2±0.13

*Dose of dicotyledons received (% body weight).
None of the hematologic values were clinically significant at $p = 0.05$.

TABLE VI

EFFECTS OF LETHAL DOSE (2% B.W.) ON
SERUM CHEMISTRIES IN CALF #1

	0 hr	1 hr	5 hrs	11 hrs*
BUN	2.4	2.8	5.2	7.2
Ca	10.5	10.2	9.8	10.1
Cl	100.0	93.0	97.0	102.0
CPK	225.0	200.0	265.0	1050.0
Gluc	95.0	99.0	203.0	8.0
Mg	2.5	2.6	2.4	3.5
Na	141.4	141.2	138.1	146.2
K	5.19	4.84	5.09	7.02
TP	7.0	6.6	6.7	7.0
SDH	- 0.1	1.5	1.8	0.6
SGGT	23.0	15.0	37.0	113.0

*Sample taken shortly after death.
BUN = blood urea nitrogen; Ca = calcium; Cl = chloride; CPK = creatinine phosphokinase; Gluc = glucose; Mg = magnesium; Na = sodium; K = potassium; TP = total protein; SDH = sorbitol dehydrogenase; SGGT = serum gamma glutamyl transpeptidase.

calf #1, the blood chemistries were narrowed to three tests because of test costs and the fact that no extreme changes in the other chemistry values occurred. The blood chemistry tests were limited to glucose, blood urea nitrogen (BUN), and serum gamma glutamyl transpeptidase (SGGT).

Serum samples were taken from calf #2 for glucose, BUN, and SGGT values. The 0 hour values were 98.0 mg/dl for glucose, 6.2 mg/dl for BUN, and 16.0 IU/L for SGGT. Serum samples taken at 9 hours post-innoculation were similar to 0 hour. Samples taken right before death were as follows: glucose 7.0 mg/dl, BUN 9.8 mg/dl, and SGGT 140 IU/L.

Calves #3 and 4's values for glucose, BUN, and SGGT remained within normal limits throughout the 1 week observation period. (See Appendix for normal values in the bovine for the above tests.)

Because calf #5, given 50 mg of CAT, was found dead, no terminal blood sample could be obtained.

The effects of lethal doses of cockleburs on blood glucose, BUN, and SGGT are presented in Tables VII, VIII, and IX. Figures 3, 4, and 5 are graphs of the mean values and standard error for glucose, BUN, and SGGT in calves dosed in Phase II versus time after exposure.

The mean $\pm$ standard error (SE) for blood glucose before clinical signs were seen was 70 $\pm$ 25.1 mg/dl. The mean $\pm$ SE after clinical signs were seen was 15.0 $\pm$ 4.3 mg/dl. The mean $\pm$ SE before the time of death was 8.5 $\pm$ 3.5 mg/dl. The mean $\pm$ SE of the BUNs and SGGTs are presented in Tables VIII and IX at various time intervals.

Serum was taken from calves #6, 7, and 9 for insulin levels. The results received are presented in Table X. The mean $\pm$ SE for the calves

TABLE VII

THE EFFECTS OF LETHAL DOSE OF COCKLEBUR ON BLOOD GLUCOSE LEVELS AT VARIOUS TIME INTERVALS

Calf #	Hour Post-Dosing (Glucose mg/dl)									
	0	5	6	7	8	10	12	14	16**	18**
6	98.0	94.0	96.0	86.0	92.0	104.0	100.0	99.0	20.0	12.0
7	102.0	101.0	--	109.0	114.0	110.0	97.0	91.0	20.0	5.0
8*	78.0	85.0	83.0	95.0	91.0	85.0	92.0	94.0	--	--
9	85.0	82.0	80.0	80.0	86.0	82.0	57.0	20.0	7.0	--
$\bar{x}$	95.0	92.3	88.0	90.0	97.3	98.6	84.6	70.0	15.0	8.5
SE	5.1	5.5	8.0	7.2	8.5	8.5	13.8	25.1	4.3	3.5

*Calf #8 died at 72 hours post-dosing. Values not used.
**Only the 16th and 18th hour samples were clinically significant ($p = 0.05$).
- = Sample not taken #7 or #8; calf #9 died at hour 15.

TABLE VIII

THE EFFECTS OF LETHAL DOSE OF COCKLEBUR ON BUN LEVELS AT VARIOUS TIME INTERVALS

Calf #	Hour Post-Dosing (BUN mg/dl)									
	0	5	6	7	8	10	12	14	16	18
6	10.6	14.2	12.2	10.6	9.6	9.0	8.6	9.2	10.2	11.6
7	3.6	5.8	--	5.0	4.2	3.0	2.2	1.8	2.8	4.8
8*	6.2	6.2	5.6	5.4	5.2	4.2	3.8	3.2	--	--
9	5.2	7.4	7.6	7.8	8.4	7.4	6.6	7.0	8.8	--
$\bar{x}$	6.4	9.1	9.9	7.8	7.4	6.0	5.8	6.0	7.2	8.2
SE	2.1	2.5	2.3	1.6	1.6	1.7	1.8	2.1	2.2	3.4

*Calf #8 died at 72 hours post-dosing. Values not used.
- = Sample not taken #7 or #8; calf #9 died at hour 15.

TABLE IX

THE EFFECTS OF LETHAL DOSES OF COCKLEBURS ON SGGT VALUES AT VARIOUS TIME INTERVALS

Calf #	Hour Post-Dosing (SGGT mg/dl)									
	0	5	6	7	8	10	12	14**	16**	18
6	25.0	21.0	17.0	23.0	15.0	22.0	67.0	199.0	118.0	127.0
7	17.0	20.0	--	17.0	19.0	22.0	23.0	122.0	185.0	243.0
8*	32.0	20.0	22.0	20.0	20.0	25.0	22.0	23.0	--	--
9	29.0	24.0	24.0	26.0	25.0	35.0	55.0	84.0	105.0	--
$\bar{x}$	23.6	21.6	20.5	22.0	19.6	26.3	48.3	108.3	136.0	185.0
SE	3.5	1.2	3.5	2.6	2.9	4.3	13.1	12.1	24.7	58.0

*Calf #8 died at 72 hours after dosing. Values not used.
**Only the 16th and 18th hour samples were statistically significant ($p = 0.05$).
- = Sample not taken #7 or #8; calf #9 died at hour 15.

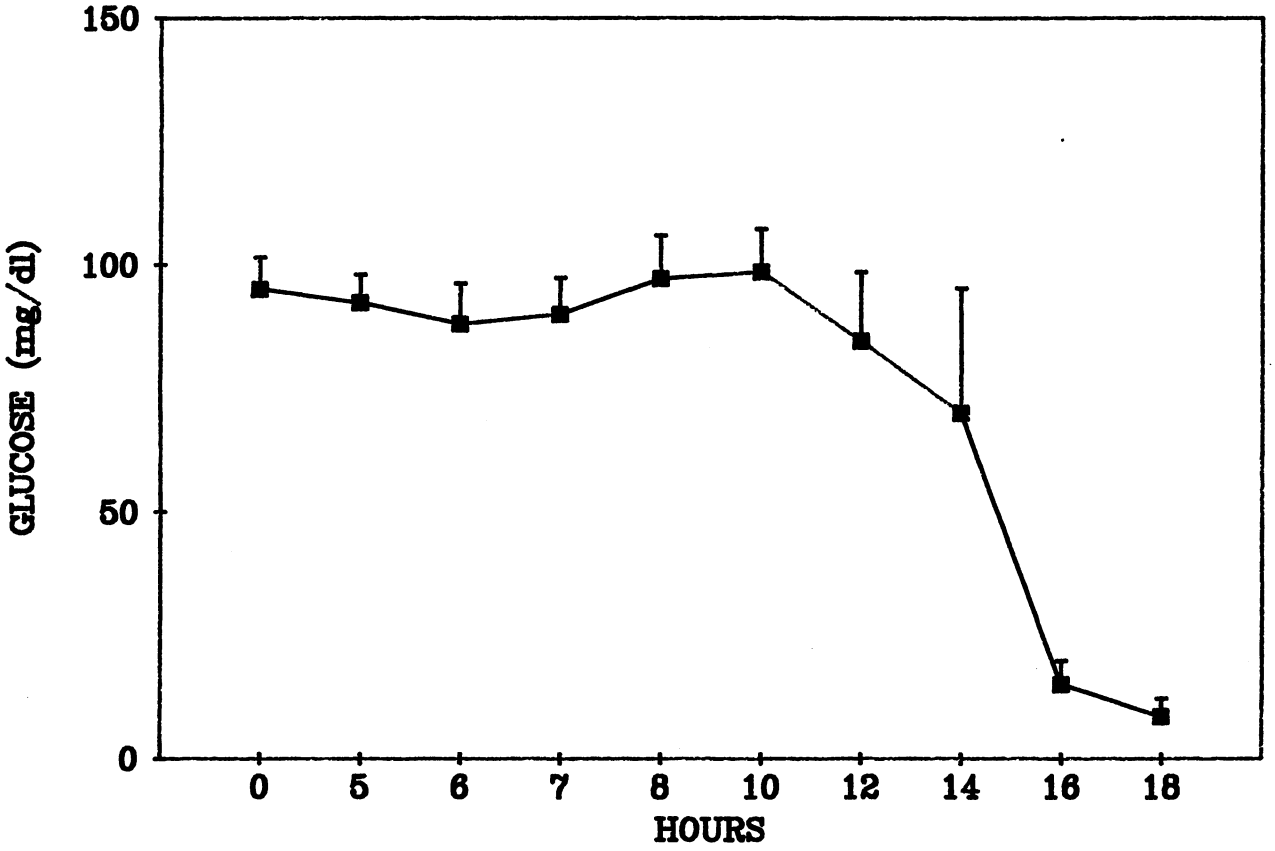

Figure 3. The Effects of Lethal Dose of Cocklebur on Blood Glucose Levels at Various Time Intervals

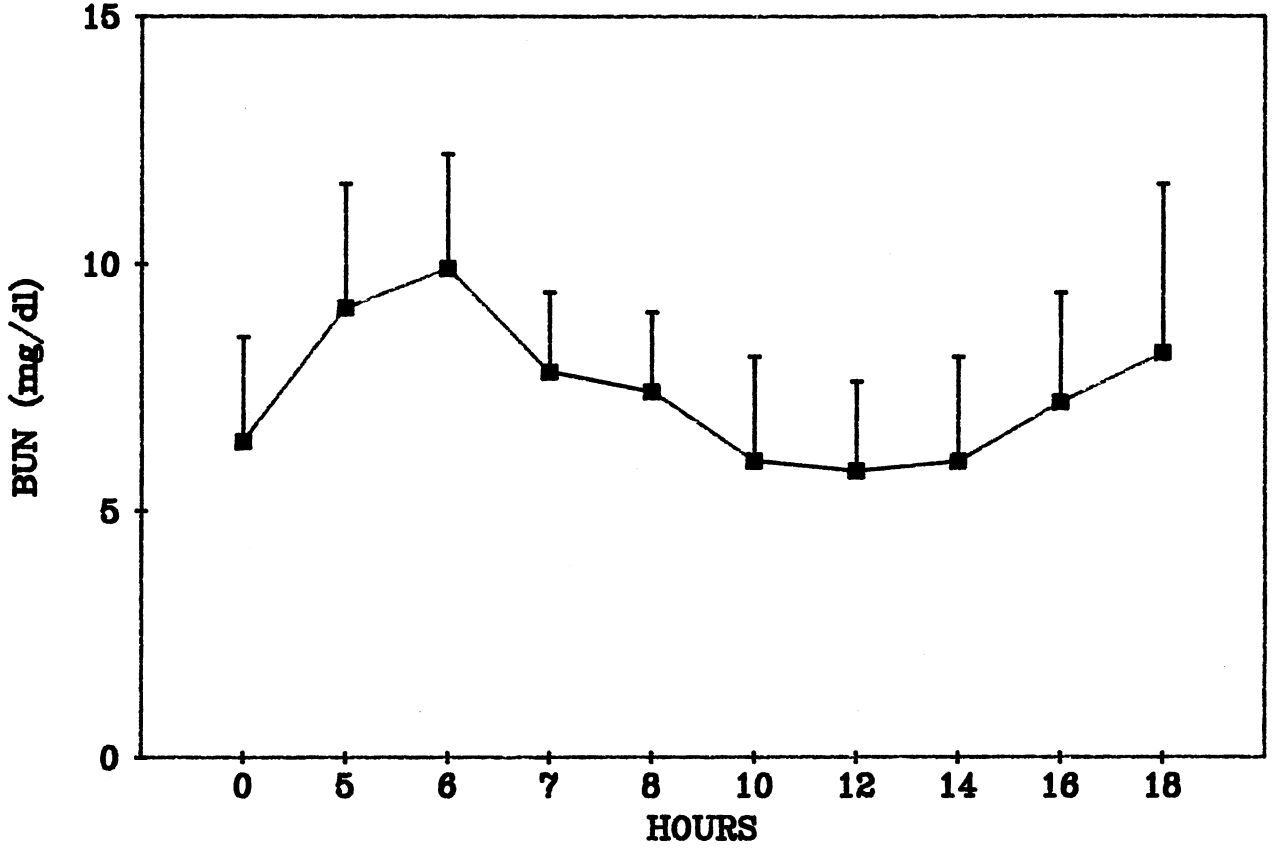

Figure 4. The Effects of Lethal Dose of Cocklebur on BUN Levels at Various Time Intervals

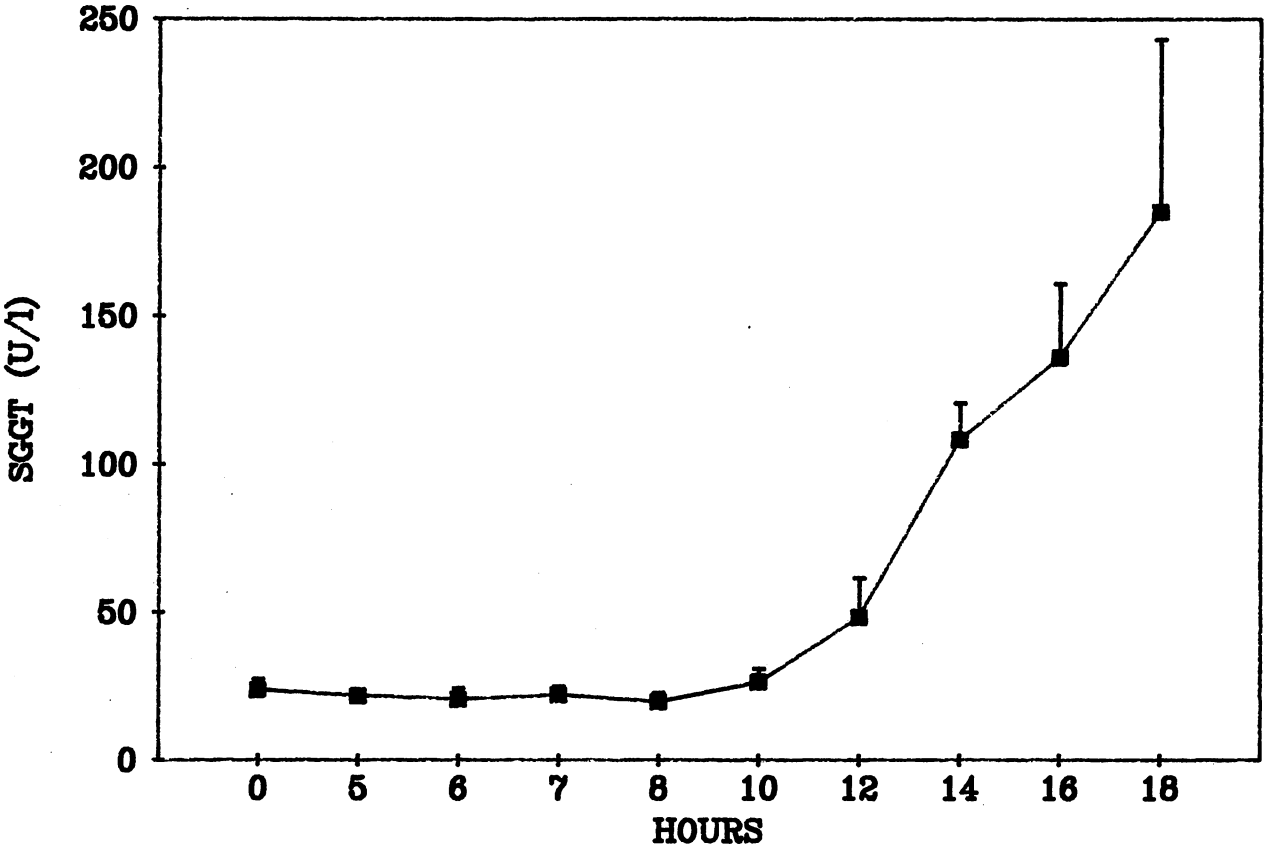

Figure 5. The Effects of Lethal Doses of Cockleburs on SGGT Values at Various Time Intervals

TABLE X

THE EFFECTS OF LETHAL DOSES OF COCKLEBURS ON INSULIN LEVELS

Calf #	Pre-Dosing Insulin Level	Insulin Levels Before Death
6	1.6 uU/ml	1.0 uU/ml
7	1.5 uU/ml	118.3 uU/ml
9	1.6 uU/ml	2.6 uU/ml
$\bar{x}$	1.56 uU/ml	40.6 uU/ml
SE	0.03	38.83

before dosing was 1.5 $\pm$ 0.033. The mean $\pm$ SE after dosing and before the time of death was 40.6 $\pm$ 38.83.

Gross Pathology

All calves were necropsied within 30 minutes of death except calves #5 and 8. Calf #5 was necropsied approximately 1 hour after death and calf #8 was necropsied approximately 2 hours after death. Calves #3 and 4 were euthanatized with a barbiturate overdose, Sleep-away[a], on post-inoculation day 7 and immediately necropsied. All calves had gross lesions except for calves #3 and 4 which received sublethal doses of cocklebur dicotyledons.

[a]Sleepaway®, Fort Dodge Laboratories, Ames, Iowa.

All calves had adequate internal body fat. No internal parasites were found in any of the calves. Except for calves #3 and 4, all calves had 300-500 mls of clear fluid in the abdominal cavity. In calf #5 the fluid clotted after exposure to air. All calves receiving a lethal dose of CAT or cocklebur dicotyledons had marked thickening of the gall bladder wall. On cut surface the walls were up to 2 mm thick due to a clear to slightly yellow gelatinous edema. The hepatic capsule and subserosa of the proximal 12-15 cm of duodenum had a similar thickening due to edema. Duodenal edema was centripetal to the bile duct.

The livers of calves #1, 2, and 5 had mild, clear edema lifting the capsule from the parenchyma. Consistently there were numerous, scattered, deep red foci 1 mm in diameter visible beneath the capsule. On cut surface the liver had an accentuated lobular pattern characterized by pale yellow and deep mahogany colored areas. There was no detectable difference between the different liver lobes.

Calves #1, 3, 7, and 9 had moderate edema in the perirenal fat. Calf #1 also had edema in the diaphragmatic wall.

Calves #7 and 9 had (diffuse or paintbrush) hemorrhages on the subepicardial surfaces. Calf #7 also had hemorrhages in the myocardium and on the subendocardial surface. In addition, this calf had 1-3 cm in diameter subserosal hemorrhages throughout the intestinal tract and subcapsular hemorrhages (2-5 cm in diameter) on the right kidney.

There were no gross lesions seen in the brains, lungs, urinary bladders, forestomachs, or intestinal mucosa of most calves. Calf #6 had nodules (1-2 mm in diameter with punctate centers) in the abomasum (nodules typical of Ostertagia).

Incidental findings included a mild, focal, dry, caseous abscessation bilaterally in the renal medulla of calf #5 and a 5 x 3 cm abscess in the urethra of calf #8.

Histopathology

All calves given lethal doses of CAT or cocklebur dicotyledons had extensive centrilobular to whole lobular hepatocellular degeneration and necrosis.

Calf #1 had extensive centrilobular hepatocellular necrosis with only a small rim of unaffected hepatocytes in peripherolobular areas. Degenerative or dead hepatocytes had a dull pink cytoplasm with nuclear pyknosis or karyorrhexis. Hepatocytes near the junctions of affected and unaffected cells had nonstaining cytoplasmic vacuoles. The hepatic sinusoids were engorged with blood (Figures 6 and 7).

Calf #2 had lesions similar to calf #1 except the sinusoids contained a larger number of neutrophils and a mild increase in the number of viable peripherolobular hepatocytes.

Calf #3 had no areas of degeneration or necrosis in the liver. Calf #4 had swollen centrilobular hepatocytes with pale cytoplasm due to clear areas containing dispersed, pink, feathery appearing cytoplasmic elements. There was no nuclear chromatin clumping or necrotic hepatocytes noted.

Calf #5 was moderately autolyzed. All hepatocytes had denser, homogeneously pink cytoplasm. There was an increased amount of neutrophils, mononuclear cells, and eosinophils in sinusoids. There were no recognizable changes of hepatocellular degeneration or necrosis.

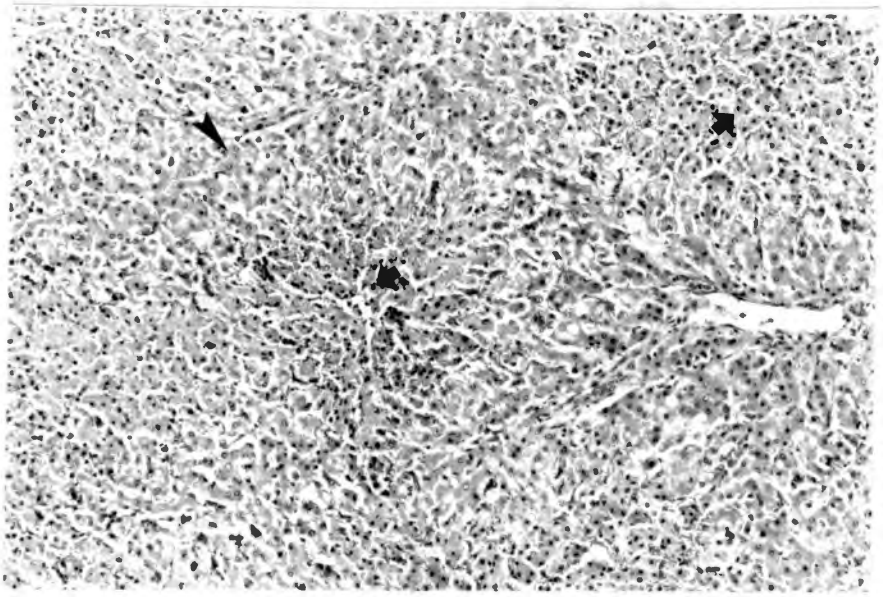

Figure 6. Photomicrograph of liver from calf given 2% of its body weight in cocklebur dicotyledons. Healthy hepatocytes in peripherolobular areas (arrowhead) and degenerative hepatocytes in centrilobular areas (arrows). X 110

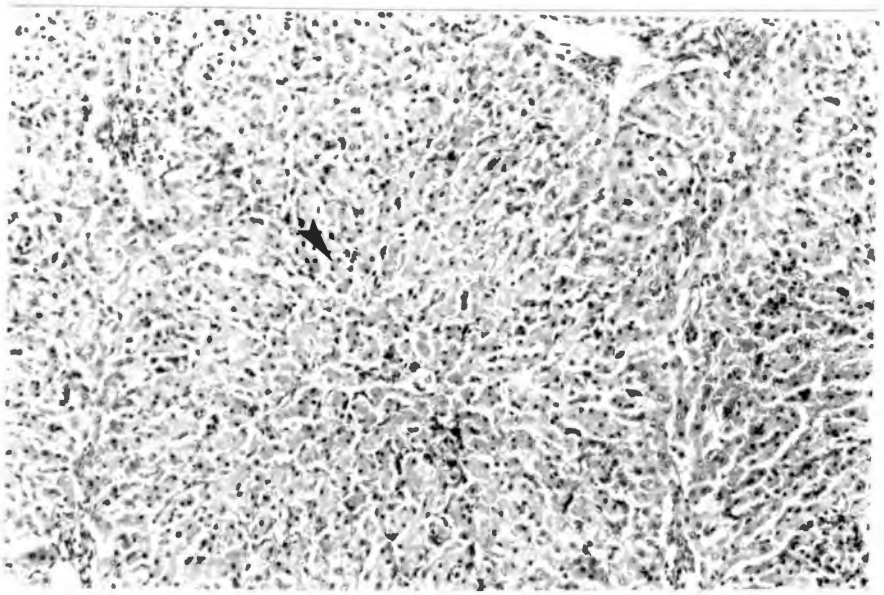

Figure 7. Centrilobular hepatocellular degeneration (arrowhead) with nuclear pyknosis. X 110

Calves #6 and 7 had similar hepatocellular lesions as calf #1. The only change that did not occur in calf #6 was severe peripherolobular cellular damage.

Calf #8 also had extensive centrilobular hepatocellular necrosis except it was not as severe as Calf #1. Centrally, there were no hepatic cords remaining, only a few hepatocytes with dense, pink cytoplasm. Most of the dead hepatocytes had been removed. There were many mononuclear cells present containing phagocytized debris (Figures 8 and 9).

Calf #9 had similar changes as seen in the livers of calves #1, 2, and 7 (Figure 10).

The gall bladder and duodenal lesions of all affected calves were similar to calf #1. Calf #1 had nonstaining edema of the gall bladder wall. There were areas where the myocytes of the muscular tunic were separated by edema. There was no vasculitis or endothelial cell enlargement noted. The duodenum had marked subserosal nonstaining edema occasionally involving the outer part of the muscular tunic. No inflammatory cell response was present in any part of the duodenum. There was some vascular engorgement of vessels in the lamina propria.

Calf #2 had similar mural edema in the gall bladder but also had aggregates of lymphocytes and scattered neutrophils around some crypts. The duodenal changes were similar to calf #1.

Calf #3 had gall bladder and duodenal lesions similar to calf #2. There were no gall bladder sections taken from calves #4, 5, and 6. Calves #7, 8, and 9 had similar gall bladder and duodenal changes as calf #2.

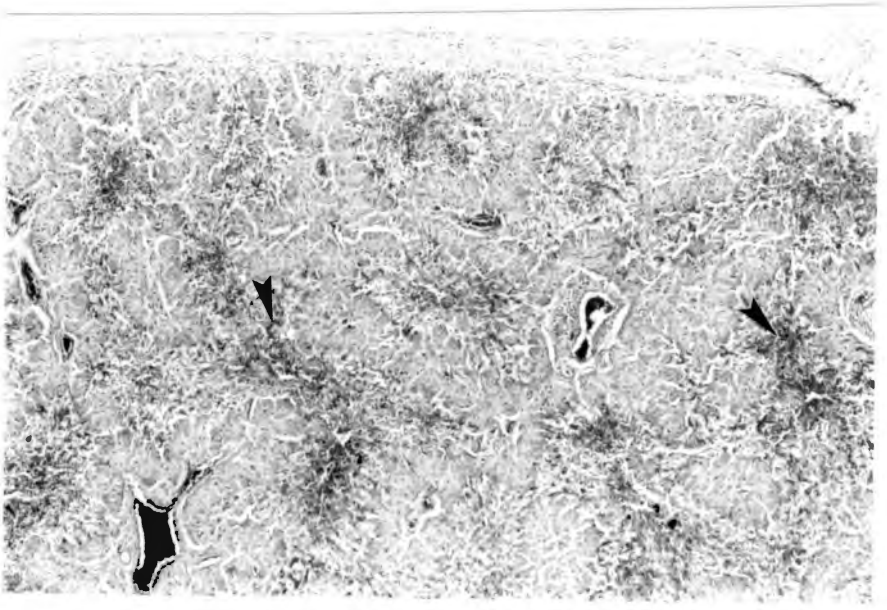

Figure 8. Photomicrograph of liver from calf surviving 72 hours after receiving 1% of its body weight in cocklebur dicotyledons. Centrilobular areas of necrosis noted by arrowheads. X 45

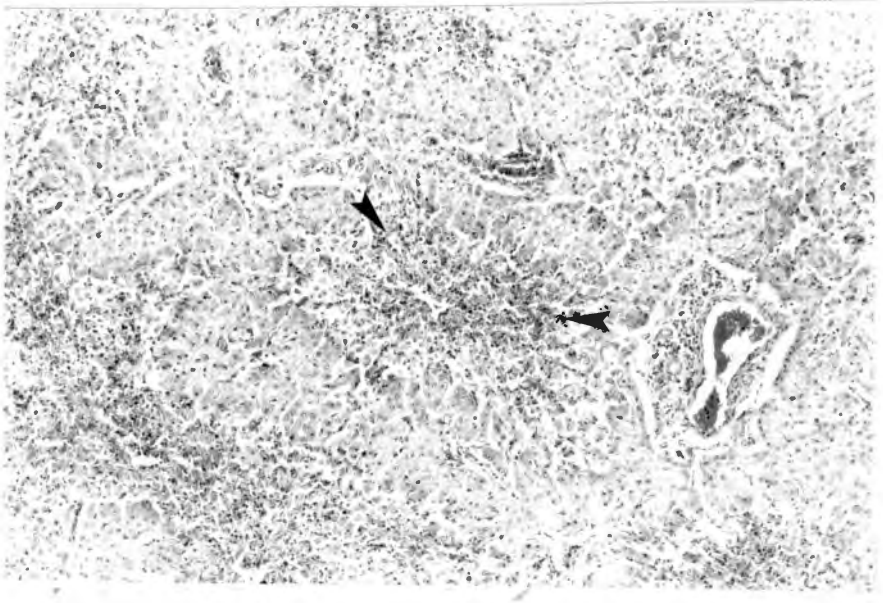

Figure 9. Higher magnification of Figure 8 showing loss of hepatic cords centrally (arrowheads). X 85

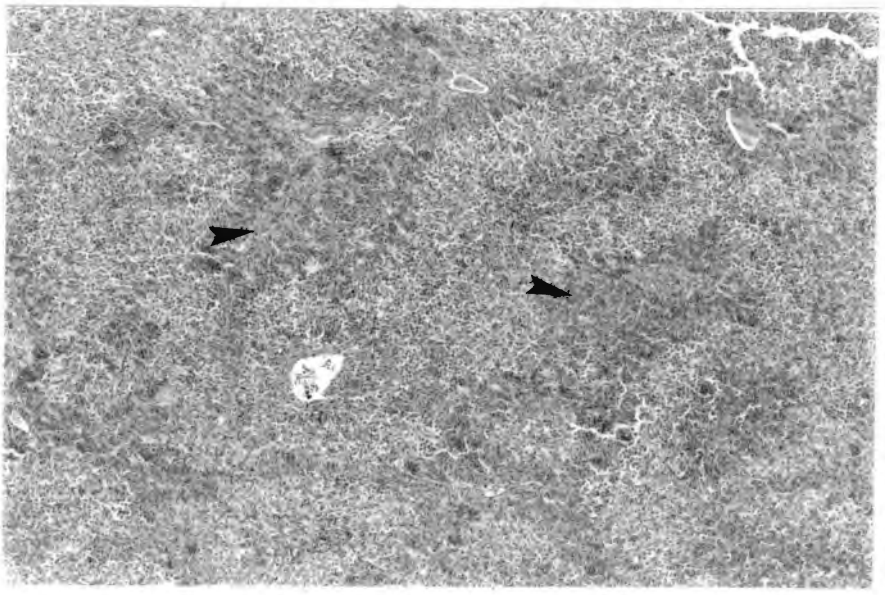

Figure 10. Photomicrograph of calf #9 living 15 hours after receiving 1% of its body weight in cocklebur dicotyledons. Marked accentuated lobular pattern due to centrilobular degeneration (arrowheads).

CHAPTER V

DISCUSSION AND CONCLUSIONS

From the observations made in Phase I, the 1% dose range was considered as the minimal lethal dose. This level is consistent with previous reports that the minimal lethal dose in cattle for cocklebur dicotyledons ranged from 1% of the animal's body weight to 2% of the animal's body weight.[1,2,5,20]

Although only four animals were used to determine the lethal dose, the numbers used gave a good indication of the dose considered to be lethal. Ideally, there should be four to five groups of animals (cattle) used with ten calves for each dose level. The normal time interval from exposure to a toxic or lethal dose of cocklebur seedlings to onset of clinical signs and death is 12-48 hours in cattle.[1,2,5] The time intervals seen are probably related to the dose received. Calf #1 died within 11 hours but it also received the highest dose of cocklebur dicotyledons (2% of body weight). The calves given the 1% minimal lethal dose of cocklebur dicotyledons died between 15-18 hours. Calf #8 lived approximately 48 hours longer than the other calves given the 1% dose level. Individual susceptibilities may explain why Calf #8 lived 2 days longer.

Previous reports in pigs indicate that death occurred within 3 to 4 hours after ingestion of a lethal dose of cocklebur dicotyledons.[2] This could explain why swine are considered to be more susceptible to the toxic effects of cocklebur dicotyledons.

Clinical signs similar to those seen in cocklebur poisoning are seen in animals with low blood levels of calcium and potassium. In hypocalcemia, animals usually have clinical signs of muscle twitching, anxious attitude, depression, anorexia, and coma leading to death. Signs seen in hypokalemic animals are neuromuscular weakness, depression, lethargy, confusion, coma, and cardiovascular changes.[21] There were no marked effects on serum calcium, potassium, magnesium, chloride, and/ or sodium in calf #1. These observations are consistent with previous reports.[3]

There were no significant changes in the hematologic values of hemoglobin, hematocrit, total protein, and WBC, in calves given lethal doses of cocklebur dicotyledons.

The clinical signs seen in the calves given a lethal dose of cocklebur dicotyledons are consistent with those reported for cattle and swine. Swine usually exhibit muscle weakness, ataxia, subnormal temperatures, spasmodic contractions of the limbs and neck muscles, rapid pulse and respiration, convulsions, and coma. Death usually occurs within 48 hours but often occurs within 3 to 4 hours.[1,2] Vomition is another common clinical sign seen in swine. Vomition did not occur in the calves receiving a lethal dose of cocklebur seedlings and is not a common sign seen in cattle. The clinical signs in swine are suspected to be due to ischemic neuronal changes and cerebral edema. These lesions have been reported in swine dying of cocklebur poisoning and have been associated with hypoglycemia.[3,22] These lesions were not seen in the calves in this experiment.

The toxin carboxyatractyloside (CAT) is known to act by physically inhibiting carrier-mediated exchange of adenosine diphosphate (ADP) and

adenosine triphosphate (ATP) across mitochondrial membranes.[23] There are subsequent decreases in mitochondrial calcium uptake, oxidative phosphorylation, cellular respiration, ATP reserves, glycolysis, glycogenolysis, gluconeogenesis, amino acid synthesis, and blood glucose concentrations.[24] Fatty acid oxidation is inhibited specifically. The clinical signs and gross and microscopic lesions seen in swine and in the rat are consistent with the proposed mechanism of action of CAT.

The clinical signs and gross and microscopic lesions seen in cattle are also consistent with the proposed mechanism of action of CAT. Since ruminants are much more dependent on volatile fatty acids, the inhibition of oxidation of these compounds by CAT may further lend to the hypoglycemia that occurred in cocklebur poisoning of the calves in this experiment.

Blood glucose levels were monitored in most calves up to the time of death or euthanasia. The values that occurred on the 16th and 18th hours post-administration of cocklebur dicotyledons were clinically and statistically significant. These values were very low ($\bar{x}$s of 15 and 8.5) and would indicate a severe hypoglycemic state. The values seen in these calves are much lower than those reported for swine even though swine poisoned with cocklebur dicotyledons were hypoglycemic. The hypoglycemia seen in swine and cattle may be the cause of the clinical signs and lesions seen in both species. In swine, ischemic neuronal changes and cerebral edema have been reported which could be associated with hypoglycemia.[3]

There are many causes of hypoglycemia in cattle and other species. Included in those are hyperinsulinemia (due to neoplasia of the pancreas or iatrogenic means), renal damage (congenital renal tubular defects or

renal toxins), starvation, malabsorption, severe liver damage due to poisons or disease, and deficient glycogen storage.[7,16,19]

Calf #7 had a marked elevation in serum insulin levels before it died. This elevation of insulin could be the cause of the hypoglycemia in this calf. Calves #6 and 9 had insulin levels within the normal range for cattle.[25] Therefore, the cause of the hypoglycemia seen could be due to a multiplicity of causes. There were no lesions observed in the pancreas of any calves receiving a lethal dose of cocklebur dicotyledons. Ultrastructural examination of beta cells of the pancreas was not done and perhaps could further elucidate the cause of hypoglycemia if done on future studies.

Histopathologic sections of the kidney were taken from all calves given lethal doses of cocklebur dicotyledons and pure CAT. There were no remarkable tubular lesions seen with light microscopy that could have caused hypoglycemia. The toxin phlorhizin is used as an additive for lubricating motor oils. Phlorhizin is known to cause glycosuria leading to hypoglycemia due to renal tubular defects.[19] Urine glucose levels were run on calf #6 (not in Results section). There were no detectable levels of glucose in the urine.

Starvation is also a cause of hypoglycemia in calves. Lack of carbohydrates and amino acids in the diet will lower blood sugar levels with time. The body then relies on fat stores for energy and then muscle. All calves in this experiment had adequate body stores of fat indicating a reasonable plane of nutrition.

None of the calves in this experiment were suffering from a malabsorption syndrome. Malabsorption is a disorder of the small intestine in which dietary nutrients are not absorbed following digestion.[21] The

animal usually suffers with chronic diarrhea, with increased fecal volume, and increased fecal fat. There were no lesions seen in the small intestine associated with malabsorption in any of the calves dosed.

Severe liver damage due to toxins or disease is another cause of hypoglycemia. The liver is one of the major organs involved in regulating glucose levels. The liver of the bovine converts proprionic acid (a volatile fatty acid produced in the rumen) to glucose. The liver also stores glucose in the form of glycogen in all species and releases the glycogen in the form of glucose when needed by the animal. In cases of severe hepatocellular damage, as seen in cocklebur poisoning, a majority of the liver may be damaged in such a manner that the glycogen is depleted and there are not enough healthy hepatocytes to convert fatty acids to glucose. This may be the cause of the hypoglycemia in cocklebur poisoning in the bovine. The calves in these experiments had severe hepatocellular damage. In most calves, there was extensive centrilobular hepatocellular degeneration and necrosis which often affected almost the entire hepatic lobule. Only a thin run of viable hepatocytes remained in peripherolobular areas. Normal cattle are somewhat glycogen deficient which may add to the complications seen in terminal cocklebur poisoning.

Gross pathologic lesions seen in the calves in this experiment are consistent with those reported in swine and cattle.[3,5,10] Previous reports of serous infiltrates in the liver, bile ducts, gall bladder wall, and duodenal wall were believed to be due to liver fluke migration since the cattle used in that study were infested with flukes.[5] The calves in this experiment were not infested with flukes but developed the same edematous changes as previously described. The edema was

probably due to vascular leakage that occurred when the hepatic cords were damaged by CAT or a metabolite of CAT.

Other than ischemic neuronal changes and cerebral edema reported in swine, histopathologic lesions seen in all calves given a lethal dose of cocklebur seedlings or pure CAT are consistent with those lesions seen in swine and rats.[3,24]

Serum gamma glutamyl transpeptidase (SGGT) levels originate from the hepatobiliary system and can be elevated five times in obstructive biliary diseases of the liver. Serum GGT levels are also elevated with hepatocellular damage as seen in this experiment. In the calves used in Phase II, on the 14th and 16th hours post-administration of cocklebur dicotyledons the values for SGGT were statistically significant. The 18th hour SGGT value probably would have been significant if there were more observations (N = 2). With two observations, the degrees of freedom were equal to 1, therefore, making the values statistically insignificant.

None of the blood urea nitrogen (BUN) values seen in the calves dosed with cocklebur dicotyledons were statistically significant as compared to the zero hour observations. This would correspond with the fact that there were no remarkable lesions seen in sections taken from the kidneys of all the calves dosed. Renal lesions are often seen in swine and rats that have been intoxicated with CAT. Renal lesions in rats consisted of distal tubular necrosis with hyaline and granular tubular casts.[26] Renal lesions seen in swine include mild to moderate acute renal tubular degeneration and necrosis. Renal lesions were characterized by increased cytoplasmic granularity and eosinophilia, and

karyolysis, and destruction of renal tubular epithelium. Hyaline and granular casts were sometimes prominent.[5,24]

CHAPTER VI

SUMMARY

Nine calves were used in this experiment to partially characterize the toxic effects of cocklebur dicotyledons in calves. Four calves were used in Phase I to determine the lethal dose of cocklebur dicotyledons. Four were used in Phase II to partially characterize the lethal effects of cocklebur dicotyledons in calves. One calf was given pure carboxyatractyloside (toxic agent in cocklebur) to insure the clinical signs and lesions seen were due to that toxin.

It was determined from the initial four calves given 2.0%, 1.0%, 0.75%, and 0.5% of their body weights in cocklebur dicotyledons that the 1% dose level was the minimal lethal dose. The four calves used in Phase II of the experiment were given 1% of their body weight in cocklebur dicotyledons. The consistent clinical signs seen in the four calves in Phase II were sternal recumbency, depression, muscle fasciculations, lateral recumbency, and paddling convulsions. The calves would usually die shortly after the convulsive seizures commenced. Three of the calves in Phase II died within 15 - 18 hours after inoculation with cocklebur dicotyledons. The other calf in Phase II died 72 hours after inoculation with cocklebur dicotyledons. Calves that were administered a lethal dose of cocklebur dicotyledons consistently had elevated SGGT levels and elevated WBC values, while having depressed glucose levels (mean 9.0 mg/dl). These same calves given a lethal dose of cocklebur dicotyledons

developed severe centrilobular to whole lobular hepatocellular degeneration and necrosis.

Hypoglycemia was believed to be the cause of many of the clinical signs observed. It was determined that there were two probable causes of the hypoglycemia. One was the severe liver damage that occurred in the calves leaving the liver functionally unable to support gluconeogenesis. The second could have been associated with hyperinsulinemia. Three out of the four calves used in Phase II were tested for predosing and post-inoculation (as close to death as possible) insulin levels. One out of the three calves had a markedly elevated insulin level on the post-inoculation sample. Hyperinsulinemia may have been a contributing factor in the hypoglycemia in that calf. Further work needs to be done to completely elucidate the causes of hypoglycemia in cattle poisoned by cocklebur seedlings.

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NORMAL BLOOD CHEMISTRY VALUES
IN THE BOVINE

	Units	Value
BUN	mg/dl	2 - 15
Calcium	mg/dl	8.9 - 11.2
Chloride	mEq/L	98 - 107
CPK	IU/L	19 - 48
Glucose	mg/dl	46 - 82
Magnesium	mg/dl	1.5 - 2.4
Potassium	mEq/L	4.0 - 6.0
Protein	gm/dl	5.0 - 7.2
SDH	IU/L	2.5 - 7.5
SGGT	IU/L	12.2 - 38.4
Sodium	mEq/L	146 - 154

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