

TOXIC EFFECTS OF MILKWEEDS (ASCLEPIAS)
IN SHEEP AND CHICKENS

By

Lanell Ogden

Bachelor of Science
Fort Valley State College
Fort Valley, Georgia
1977

Doctor of Veterinary Medicine
Tuskegee University
Tuskegee, Alabama
1981

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1989

Thesis
1989
0344
cap. 2

TOXIC EFFECTS OF MILKWEEDS (ASCLEPIAS)

IN SHEEP AND CHICKENS

Thesis Approved:

George E. Benson

Thesis Advisor

Sulhuah Sayyah

Ronald J. Jyd

Norman N. Durham

Dean of the Graduate College

PREFACE

This study describes the toxic effects of several Asclepias species in sheep and chickens. Clinical signs, electrocardiographic changes, rectal temperature, and respiration rate were monitored in sheep. Clinical signs, body weight, and feed consumption were recorded in chickens. Gross and histopathological examinations were conducted in both chickens and sheep. The results obtained further characterize the toxic effects and clinical syndromes produced by Asclepias especially several of the less well studied species. Furthermore, this study illustrates that chickens can serve as animal models in screening livestock toxicants.

The author expresses appreciation to Dr. George Burrows for guidance and assistance during this study. Appreciation is also extended to committee members, Dr. Ron Tyrl, Dr. Sabbiah Sangiah, and Dr. William Edwards. Thanks are given to Dr. Ray Ely and Dr. Sam Gorham for assistance in pathological evaluations. Special gratitude is extended to Dr. Dan Goodwin and the Oklahoma Animal Disease Diagnostic Laboratory for support and assistance throughout this project. Appreciation also is conveyed to the staff at Oklahoma State University Poultry Research Center for support and assistance in conducting the poultry section of this study. Thanks are given to Dr. Earl Mitchell for spending several weeks making reagents to conduct the analytical portion of this study. Thanks to Dr. Everett Short and Johnny Johnson for assistance with the manuscript and guidance on the use of the computer.

A note of thanks must go to individuals that assisted in field collections of Asclepias (Dr. Sandra Morgan, Dr. Fahd Eissa, Ms. Belinda Green, Dr. Susanne Short, Ms. Deanna Bohlen). Special thanks are offered to Ms. Staci Smith for assistance in stuffing all those gelatin capsules with ground plant material. Finally, a special thanks to my husband and best friend for support, patience, understanding, and words of encouragement offered during this stage of my career.

TABLE OF CONTENTS

Chapter	Page
I. THE RESEARCH PROBLEM	1
II. REVIEW OF THE LITERATURE	3
Asclepiadaceae	3
Introduction	3
Ecological Chemistry	3
<u>Asclepias</u>	4
Introduction	4
Habitat	5
Uses	6
Chemistry	7
Mechanism of Toxin Action	8
Intoxications in Sheep and Goat	11
Intoxications in Horses and Cattle	13
Intoxications in Chickens and Turkeys	14
Intoxications in Rabbits	15
Pathological Changes and Treatment	16
III. MATERIALS AND METHODS	17
Plant	17
Experiments in Sheep	18
Experiments in Chickens	20
Feeding Studies	20
Dosing Studies	20
Postmortem Examinations	20
IV. RESULTS	22
Experiments in Sheep	22
Clinical Observations	22
Electrocardiography	24
Clinical Pathology	24
Gross Pathology	25
Histopathology	26

Chapter		Page
	Experiments in Chickens, Feeding	31
	Clinical Observations	31
	Feed Consumption and Body Weight Change	32
	Gross Pathology	33
	Histopathology	33
	Experiments in Chickens, Dosing	33
	Clinical Observations	33
	Feed Consumption and Body Weight Change	35
	Gross Pathology	37
	Histopathology	37
V.	DISCUSSION	40
VI.	SUMMARY	45
VII.	BIBLIOGRAPHY	47

LIST OF TABLES

Table	Page
I. Comparative Toxicity of Several Milkweed Species Reported in Sheep	12
II. Plant Collection and Identification	17
III. Dosage of Sheep with <u>Asclepias</u> , % Body Weight, Weight and Plant Parts	19
IV. Histopathological Observations in Sheep given <u>Asclepias</u>	27
V. Summary of Dose and Effect of <u>Asclepias</u> in Sheep	30
VI. Mean Consumption, Body Weight Change, Mortality of Chickens Fed <u>Asclepias</u>	32
VII. Mean Consumption, Body Weight Change, Mortality of Chickens Dosed with <u>Asclepias</u>	36

LIST OF FIGURES

Figure	Page
1. Structure of <u>Asclepias</u> Cardenolides	10
2. Pulmonary edema in Sheep given Oral <u>Asclepias</u>	28
3. Myocardial Degeneration; Infiltrations of Macrophages and Lymphocytes in the Myocardium of Sheep given <u>A.</u> <u>subverticillata</u>	29
4. Infiltrations of Macrophages and Heterophils in the Myocardial Interstitialium of Chickens given <u>A. verticillata</u>	39

CHAPTER I

THE RESEARCH PROBLEM

Species of the genus Asclepias vary in height, leaf width, flower color, root type, presence of a latex sap, cardenolide content, type of cardenolide, and epithelial appendages. The unique morphology of the flower with its corona and androstigimium is, however a consistent and distinctive feature of the genus. Although Botanists and entomologists recognize the great morphological diversity among species of Asclepias, little consideration is given by toxicologists to the possibility of different intoxication syndromes being produced by different species. Toxic effects of the genus are typically attributed to the commonly encountered cardiac glycosides. Although the presence of cardiac glycosides has been confirmed in many species, other toxicants may be present and an array of syndromes may be produced by different species of Asclepias. Asclepias-intoxicated animals present the veterinarian with diverse clinical manifestations which must be treated symptomatically to prevent death. Knowledge of these different syndromes and the toxic manifestations of less-known species may prove helpful in treatment and or prevention of intoxications.

The purpose of the study is to better define differences in toxicity between A. verticillata and A. subverticillata; to describe the clinical manifestations of intoxication including several of the less well known species of Asclepias, and to compare toxic effects of

Asclepias species in sheep and chickens using clinical signs, pathological lesions, and mortality as parameters.

CHAPTER II

REVIEW OF THE LITERATURE

Asclepidaceae

Introduction. The milkweed family, Asclepidaceae, is a well-defined group of dicotyledonous plants characterized by pollen adhering in a waxlike mass, by a milky sap, and by follicles (pods) with tufted seeds. The family includes about 200 genera and 2500 species of herbs, vines, or shrubs. The leaves are simple, alternate, opposite or whorled, and exstipulate. The flowers are perfect, regular, and arranged primarily in umbels(Pammel, 1911; Woodson, 1954).

Medicinal uses of Asclepidaceae have been documented for centuries. Various species were used in treatment of warts, bronchitis, cancer, heart disease, and also as emetics, tonics and diuretics(Pammel, 1911). The toxic nature of the milkweeds was well recognized by ancient African warriors who used the latex of Calotropis procera as an arrow poison(Mahmound et al., 1979b); Seiber et al., 1983; Pahwa, 1988). Some species have been cultivated as ornamentals, while others have been used for latex production, and some species have been considered possible energy sources(Emon et al.,1985).

Ecological Chemistry. As early as the mid 1800's, naturalists observed that birds avoided eating certain butterflies whose larvae fed on species of Asclepidaceae. Birds that ate glycoside-containing butter

flies vomited shortly thereafter, thus as a consequence, learning to avoid certain butterflies. The so-called gourmand-gourmet hypothesis was proposed to describe this form of conditioning behavior in birds. The hypothesis was later extended to include butterfly mimicry (Brower, 1969; Smith, 1979). Representatives of at least eight orders of insects feed upon the Asclepidaceae and Apocynaceae (Rothschild, 1973). Larvae of the monarch butterfly (Danaus plexippus), the milkweed bug (Oncopeltus fasciatus), and the North African grasshopper (Poekilocerus bufonius), feed upon Asclepias species and store cardenolides for defensive purposes (Brower, 1968, 1969, 1978; Isman, 1977). The grasshopper and milkweed bug deploy the poisons in defensive secretions while in the monarch butterfly, tissue incorporation renders the insect unpalatable to avian predators throughout all life stages (Euw et al., 1967; Brower et al., 1974, 1978; Enson et al., 1978; Seiber et al., 1985).

Asclepias

Introduction. Asclepias is the predominant genus of Asclepidaceae in North America and is composed of 108 species which are found from Mexico throughout virtually every state in the U.S., and extending into Canada (Woodson, 1954). Asclepias species are most common in the central plains and Western U.S. and Canada. In the U.S., the plants are most abundant from Utah and Wyoming to Texas and Mexico. Among the 26 species of Asclepias recognized in Oklahoma, information regarding toxicity is reported only for 10 species (Edwards et al., 1984).

Members of the genus Asclepias are erect single-stemmed, summer and autumn flowering perennials characterized by a unique flower, a pod-like fruit, and whorled or opposite leaves of various widths. The unusual flowers are clustered in terminal or axillary umbels. The

corolla consists of five petals which are strongly reflexed. Extending upward from the base of each petal is a club-shaped or hooded lobe. Collectively, the five lobes are referred to as the corona. Flower colors range from a greenish, creamy white to orange to pink or purple. The fruit is an inflated follicle filled with numerous seeds, each with a tuft of long silky hairs. Species of Asclepias may be divided on the basis of leaf width into narrow, intermediate and broad-leaf groups. In one classification(Edwards et. al,1984), the leaves are considered narrow if less than a centimeter wide(e.g. A. subverticillata, A. verticillata), intermediate when 1-4 cm wide(e.g. A. tuberosa, A. viridis) and broad if more than 4 cm in width(e.g. A. latifolia, A. speciosa, A. syriaca). Kingsbury(1964) classified species as broad if leaves exceeded 1.5 inches and narrow leaved if leaves were less than 1.5 inches in width.

Habitat. Species of Asclepias may be found in irrigation ditches, fence lines, prairies, borrow ditches, waterways, open woods, and marshy areas. Examples of the diversity in habitat include; A. incarnata which is found in marshy areas of the eastern U.S., A. exaltata which occurs mainly at forest borders, and A. amplexicaulis which is found on sandy shores or associated with alluvial deposits. These restricted habitats are in contrast to that of A. speciosa, which is widely distributed in various environments(Woodson,1954). Most species occupy open sites although some including A. incarnata, A. lanceolata, A. purpurascens, A. speciosa, A. syriaca, A. tuberosa, A. variegata, A. verticillata and A. viridiflora are found in partially or fully shaded areas(Edwards et al.,1984).

Uses. The indigenous inhabitants and early settlers of America and Africa made extensive use of Asclepias species, especially phloem fibers of the stems and the hairs of the seed known as coma. The seed floss of A. syriaca was used as a stuffing for pillows and cushions by colonists of New England. The stem fibers of Asclepias, particularly those of A. incarnata and A. syriaca, have been proposed as a substitute for flax and hemp although generally considered inferior to them. During World War II, seed floss proved to be the best substitute for kapok in the manufacture of life jackets for the U.S. Navy, and 150,000 lbs were harvested in 1944. Both the stem fiber and the floss have been advocated as stock for paper making. Secondary products of potential value include cellulose from the stems and a semidrying oil from the seed. Milkweed latex was once considered a likely source of rubber in temperate latitudes. Biochemical investigations, also, were conducted to assess the value of the latex of certain species for use as an oral contraceptive(Woodson, 1954). Other uses include: A. tuberosa(pleurisy-root)as a diuretic and an emetic(Sayre et al., 1899; Milks et al., 1943) and A. currasavica as a vermifuge and in the preparation of glue (Pammel, 1911). Asclepias tuberosa and A. curassavica are frequently used as ornamentals (Edwards et al., 1984; Woodson, 1954). The cytotoxic character of Asclepias curassavica led to investigations of its use as an anticancer agent(Kupchan et al., 1964).

Chemistry. The first attempts to characterize the toxic principle of A. eriocarpa were reported by Couch (1929). A nonalkaloidal, nonglycosidal resinoid was isolated from A. eriocarpa which was lethal to guinea pigs. However, in a subsequent study (Seiber et al., 1983) investigators were unable to reproduce toxicity in mice using a similar procedure of extraction and purification from A. eriocarpa (Seiber et al., 1983).

Asclepias species contain cardenolides, which are C₂₃ steroids. These 5 α cardenolides are characterized by the presence in the "genin" (- aglycone, R=H) of (1) α , -unsaturated γ lactone (butenolide) ring attached at C-17, (2) a cis juncture of rings C and D, and (3) a 14 -hydroxy group. Important characteristics differentiating the Asclepias cardenolides from those of other genera include changes in the configurations at C₃, C₅, C₁₇ and oxygenation patterns (usually hydroxy or carbonyl O) at various positions from among C₁, C₂, C₅, C₁₁, C₁₂, C₁₅, C₁₆, and C₁₉. Some cardenolides have an additional olefinic double bond, and others have an epoxy group in the steroid ring. Cardenolides usually occur in nature as glycosides (cardiac glycosides), attached generally through an OH at C₃ (C₁, C₂, and C₁₁ are other positions of glycosidation) to one or more sugar moieties. Over 20 different sugars have been isolated from hydrolysis of the cardenolides of Asclepias, but only three of these sugars are known to occur in other classes of natural products. A common pattern is for the genin to be attached to one or more rare sugars, and then to one or more glucose molecules as is seen in digitalis cardenolides. Unless enzymatic hydrolysis is inhibited during the extraction and isolation procedures, the glycosides may retain only the rare sugars (Seiber et al., 1978, 1983,

1984).

The configuration at the A:B ring juncture primarily distinguishes cardenolides of the Asclepidaceae from the clinically useful cardenolides of the Apocynaceae and Scrophulariaceae. The Apocynaceae has the 5 α (trans A/B) and the Scrophulariaceae has the 5 (cis-A/B) configuration. For example, uzarigenin is widely distributed in the Asclepidaceae while its 5 isomer, digitoxigenin, is found in the Scrophulariaceae but not in the Asclepiadaceae. In addition, a number of milkweed cardenolide genins have hydroxy groups at C₂ and C₃, both of which may be involved in a cyclic bridge to a single sugar moiety. This feature, which is not found among the clinically useful cardiac glycosides, produces cardenolides which are markedly resistant to acid hydrolysis. Chemical analyses of Asclepias species have revealed the presence of several different cardenolides including simple genins and at least two groups of 2,3-dihydroxy cardenolide derivatives with cyclic bridged sugars. The glycosides uzarigenin, desglucouzarin, syriocide, and syriobioside have been isolated from A. syriaca. Labriformin, eriocarpin, and labriformidin glycosides have been isolated from A. labriformis and A. eriocarpa. A. speciosa contains desglucosyriocide and several additional cardenolides in common with A. syriaca but lacks labriformin and labriformidin, as does A. syriaca (Seiber et al., 1983).

Mechanism of Toxin Action. The mechanism of action of Asclepias cardenolides as well as the therapeutic cardiac glycosides digoxin and digitoxin is believed to be inhibition of the Na⁺ - K⁺ ATPase enzyme system. Despite structural differences, cardenolides found in Asclepias resemble ouabain in their ability to inhibit Na⁺ - K⁺ ATPase [I 50 %]

activity (Benson et al., 1978, 1979).

Cardenolides may not be the primary toxic principle in all milkweed poisonings. The partially characterized resinoid, galitoxin isolated from A. subverticillata and A. mexicana, produced effects similar to those seen in guinea pigs and sheep that were administered plant material of A. subverticillata (Marsh et al., 1920). Because of this, early researchers considered resinoids responsible for the production of the spasms seen in Asclepias poisonings (Kingsbury, 1965). Furthermore, some of the more toxic species of Asclepias, such as A. subverticillata and A. mexicana, have been shown to contain little or no cardenolides (Seiber et al., 1985). However, most species of Asclepias contain at least a small quantity of cardenolides. Some of the differences in effects may be due to the variation in type, proportion and concentration of cardenolides in Asclepias species (Seiber et al., 1983). Furthermore, a positive correlation has been found between the cardenolide content of four milkweed species, A. labriformis, A. eriocarpa, A. speciosa and A. fascicularis, and their relative toxicity to mice. The order of cardenolide content and toxicity of the plants was A. labriformis > A. eriocarpa > A. speciosa > A. fascicularis. In addition, after extraction of A. eriocarpa and subjection of the extract to solvent partition clean-up and column chromatography, the toxicity of the phases or column fractions to mice increased as the cardenolide content increased (Seiber et al., 1983).

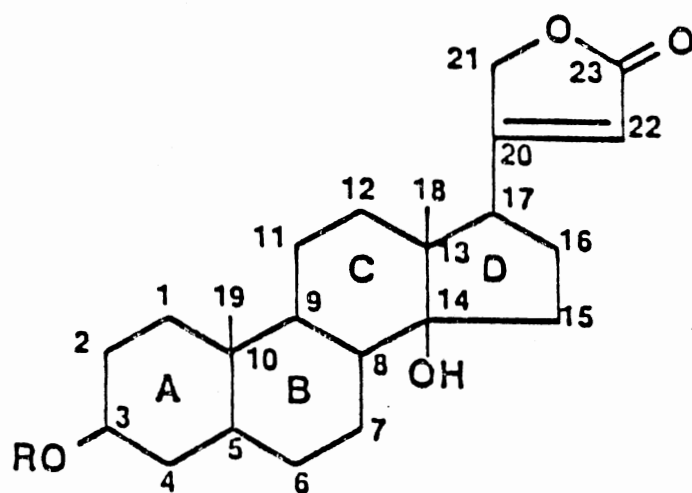


Figure 1. Structure of Asclepias cardenolides

Intoxications in Sheep and Goats . Milkweeds are generally not palatable, therefore, they are not readily consumed unless there is no other available forage(due to overgrazing of the range) or there are considerable amounts intermixed in hay.(Kingsbury, 1965; Sprawls, 1982; Seiber et al., 1985).

Devastating losses of sheep in Colorado in the early 20th century directed attention toward Asclepias as highly toxic plants. Losses of 350 and 750 ewes were reported from flocks of 1700 and 1400 animals that were held for 24 hours or less in fields containing large numbers of A. subverticillata (Glover et al., 1915, 1917).

Poisoning by milkweeds seems to occur most frequently in sheep and goats. Of the Asclepias species studied, most are lethal at 2 % or less of the animals body weight of the green plant. Signs of poisoning in sheep due to A. subverticillata, A. verticillata or A. pumila (all narrow-leaf species)include spasmodic chewing movements of the jaws, jaw champing, twitching of the eyes and ears, muscular weakness(especially marked in the hind legs), staggering, weak and rapid pulse, labored respiration(expiratory grunts), dilated pupils, elevated temperature, bloat, spasms with opisthotonos, and running movements. Signs observed in sheep after consumption of broad-leaf species(A. eriocarpa, A. latifolia, A. speciosa)include: depression, excessive salivation, abdominal pain, rapid weak pulse, labored respiration, bloody-mucoid diarrhea, and hypothermia. Spasms were not part of the clinical syndrome; and animals died quietly(Tunnicliff et al, 1930; Rowe et al., 1970).

TABLE I

COMPARATIVE TOXICITY OF SEVERAL MILKWEED SPECIES REPORTED IN SHEEP*

<u>Asclepias</u>	Minimum toxic dose	Minimum lethal dose
<u>species</u>	(lb/100-lb sheep)	(lb/100-lb sheep)
<u>A. labriformis</u>	-	0.05-0.2
<u>A. eriocarpa</u>	0.1-0.2	0.22
<u>A. subverticillata</u>	0.22	0.22
<u>A. pumila</u>	0.79	2.16
<u>A. mexicana</u>	0.88	1.32
<u>A. verticillata</u>	2.20	-
<u>A. speciosa</u>	2.60	-

*Green plant

(Seiber et al., 1985).

Intoxications in Horses and Cattle. Horses intoxicated with A. subverticillata showed signs of colic, intermittent brief periods of lying down, uneasiness, incoordination, weakness of the hind quarters, trembling, periodic falls, mydriasis, marked sweating and convulsions. Death occurred subsequent to respiratory failure. Intoxications and death of 450 kg horses have been reported to occur with ingestion of as little as 1.0 kg or less of green plant. Clinical signs of A. subverticillata poisoning in cattle, while resembling that in horses, usually begins with depression, muscle fasciculations and progressive weakness. Eventually these progress to include incoordination, convulsions, bloat, grunting noises, respiratory collapse, and death (Marsh et al., 1920; Sprowls, 1982; Edwards et al., 1984).

Almost all of a herd of 962 heifers were reported to be ill the day after consumption of maize silage that contained 40-45 % A. syriaca (Gabor et al., 1987). Signs of intoxication included inappetence, listlessness, ruminal stasis, dyspnea, mild bloody diarrhea, oral and nasal discharge, and ocular disturbances. Death occurred in 11 of the 36 most severely affected heifers within six days post exposure. Alveolar and interstitial emphysema were the most significant postmortem findings. An experimental study was conducted in adult sheep to confirm the toxicity. Approximately 30 grams of powdered A. syriaca seeds was administered by ruminal fistula. Signs observed included a slowing of the heart rate from 84 to 58 per minute which was followed by a compensatory increase from 52 to 104 per minute. During the next 24 hours after exposure, sheep displayed bruxism, dyspnea, and ruminal stasis, with moderate bloating.

Intoxications in Chickens and Turkeys. A. mexicana caused a 50 % mortality in a flock of 700 eight-week old white leg horn pullets. Signs included lameness, twisted neck and loss of motor control (Campbell, 1931). Experimental reproduction of the clinical syndrome to confirm the toxic nature of A. mexicana resulted in similar signs including severe incoordination, violent convulsions, abnormal posture with leg and wing movements, torticollis, and collapse. Survivors gradually recovered during the three days following onset of signs. There were no abnormalities observed upon necropsy of affected chickens (Campbell, 1931).

Several turkey producers in the vicinity of La Junta, Colorado reported incidences of intoxication of turkey poults that consumed A. subverticillata. Approximately 10 % of one flock had spasms within two hours after exposure and died a few hours later. Several young poults from another flock of 200 had convulsions and incoordination less than an hour after consumption of young tender shoots of A. subverticillata. Daily losses of 10 to 15 poults ultimately resulted in a 20 % mortality in the flock. Consumption of A. subverticillata resulted in 39 deaths among a flock of 950 half-grown turkeys. Incoordination and staggering occurred in 35 other turkeys in the flock and nearly all affected turkeys died within a few days.

Experimental studies in chickens and turkeys produced in high mortality and morbidity in both species. Young chickens that were dosed at 1 % body weight of green A. subverticillata exhibited lameness and convulsions. Asclepias subverticillata that was fed to three week old turkey poults at a dose of 1 gram of green plant/100 grams of body

weight caused spasms and death within five hours. Clinical signs were categorized into four stages (Stiles, 1942). The prodromal stage was associated with drowsiness, listlessness, irregular movements, and a lack of convulsions. During the second stage, observations included convulsions, loss of muscular control of legs and wings, falling backward like a pouter pigeon, lying on the back and sides with flapping wings and trembling legs, clonic intermittent seizures, twisted head, arched neck, dyspnea, dilated pupils and inability to stand. The third stage was characterized by highly visible running movements of the legs and tremors of the wings and eyelids. An unusually rapid heart rate and spasms could be triggered upon arousal. Finally, the fourth rapid stage involved less severe spasms, exhaustion, coma, respiratory paralysis, and death. The temperature decreased by several degrees by the time of death. Necropsy examination revealed crops distended with a small amount of gas mixed with mucus. There were no significant histopathological lesions in the brain, liver, kidney, and heart (Stiles, 1942).

Asclepias intoxication in chickens has also been associated with depression and atony of the crop and gizzard (Kingsbury, 1964).

Intoxications in Rabbits. Symptoms of milkweed poisoning were seen in 18 of 65 rabbits which became paralyzed after consumption of baled wheat straw that was contaminated with A. eriocarpa (Vail, 1942). Affected rabbits were paralyzed in various postures, suffered loss of control of the cervical musculature, and had various degrees of anterior or posterior paralysis. Other signs included an abnormal quantity of urine, excessive salivation, hypoesthesia, hypothermia, tar-like feces, anorexia, cyanosis, weakness, coma and death. The toxicity of A. eriocarpa in

rabbits was confirmed experimentally (Vail, 1942).

Pathological Changes and Treatment. Pathological findings are similar in most animal species exposed to Asclepias. Only a few, nonspecific pathological changes have been associated with narrow-leaf species including agonal hemorrhages in the trachea, lungs and myocardial surfaces. The kidney, lung, and central nervous system may be congested and the urinary bladder is generally empty. Changes which are typically associated with the broad-leaf species include hemorrhage and inflammation of the gastrointestinal tract and congestion of the kidney, liver, and lung (Benson et al., 1978; Edwards et al., 1984).

There is no specific antidote, however, symptomatic therapy may include sedatives for control of convulsions and atropine and/or other antiarrhythmic agents for the cardiac effects (Clark, 1979). Prevention is the best approach, however, this may be difficult since these plants are difficult to eradicate and their growth is patchy.

CHAPTER III

MATERIALS AND METHODS

Plant

Ten species of Asclepias were collected while in flower during late spring and summer from various localities in Oklahoma, Arizona, Utah, Kansas and Missouri. Plants were identified, labeled, and air dried. Pods were removed prior to grinding plants in a Wiley Mill. Ground plant material was stored at 28.9° C until used. One specimen from each collection site was pressed, dried and stored for confirmation of identity and reference (Table II).

TABLE II

PLANT COLLECTION AND IDENTIFICATION

<u>Asclepias</u> Species	Collection Site	Growth Stage	Flowers
<u>A. viridis</u>	Stillwater, OK	Pods	present
<u>A. syriaca</u>	Great Plains, OK	Pods	none
<u>A. speciosa</u>	N.W., MO	Pods	whole
<u>A. tuberosa</u>	S.W., OK	no pods	present
<u>A. labriformis</u>	Utah	Pods	present
<u>A. verticillata</u>	Kansas	few pods	present
<u>A. subverticillata</u>	Arizona	no pods	present
<u>A. incarnata</u>	Kansas	no pods	present
<u>A. hirtella</u>	Kansas	no pods	present
<u>A. latifolia</u>	Oklahoma	Pods	none

Experiments in Sheep

Eleven, apparently normal, adult, female, crossbreed white face sheep were individually housed in pens at least 48 hours prior to beginning the experiment and sheep were maintained on commercial pellets, and alfalfa hay. Environmental temperature ranged from 20 - 22 C with a relative humidity of approximately 40- 60 %. Physical examinations and preexposure electrocardiographic recordings were conducted and preexposure blood samples were obtained from each sheep. At twenty four hour intervals and during clinical episodes blood samples was obtained and electrocardiographic recordings were performed on each sheep after exposure.

Hematological parameters evaluated included total white blood cell count, total red blood cell count, hemoglobin(Hb), mean corpuscular hemaglobin(MCH), mean corpuscular hemoglobin concentration(MCHC) and total protein concentration. The following serum chemistry parameters were determined; total bilirubin, blood urea nitrogen(BUN), creatinine, Lactate dehydrogenase(LDH), creatine phosphokinase(CPK), total protein, serum aspartate aminotransferase(AST), gamma glutamyl transferase(GGT). Serum sodium, magnesium, phosphorus, potassium, and chloride levels were also determined.

Sheep were fasted overnight and dosed approximately every 24 hours at various doses according to Table II. Crude plant material was mixed with water and administered to sheep by use of a ruminal tube and a stomach pump. Signs, rectal temperature, respiration rate, and electrocardiographic changes were recorded.

TABLE III

DOSAGE OF SHEEP WITH ASCLEPIAS, % BODY WEIGHT, WEIGHT AND PLANT PARTS

<u>Asclepias</u> species	Dose* % BWT	Weight Kg	Plant parts
<u>A. viridis</u>	0.2	74	Stems, Leaves
<u>A. syriaca</u>	0.5	66	Stems, Leaves
<u>A. speciosa</u>	0.5	50	Stems, Leaves
<u>A. tuberosa</u>	0.5	55	Stems, Leaves
<u>A. labriformis</u>	0.1	51	Stems, Leaves
<u>A. verticillata</u>	0.2	50	Stems, Leaves
<u>A. verticillata</u>	0.4	51	Stems, Leaves
<u>A. subverticillata</u>	0.2	50	Stems, Leaves
<u>A. incarnata</u>	0.5	53	Stems, Leaves
<u>A. hirtella</u>	0.5	53	Stems, Leaves
<u>A. latifolia</u>	0.2	66	leaves only

*dry weight of plant

Experiments in Chickens

Seven week old male White leghorn chickens with a mean weight of 2 kg were obtained from Oklahoma State University Poultry Research Facility. Chickens were fed a commercial poultry ration and were individually housed in stainless steel wire hanging cages that were supplied with municipal water by an automatic watering system.

Statistical Analysis. Preexposure body weights and postexposure body weights of chickens were analyzed by the Paired Student's T Test for significant difference using $p \leq 0.5$ and $p \leq 0.1$ for determinations of significance.

Feeding Studies. A seven-day feeding study was conducted using 4-8 chickens per group. Experimental groups were given a 50 % mixture of commercial poultry feed and finely ground A. tuberosa, A. viridis, A. syriaca, A. speciosa, A. labriformis, A. verticillata or A. subverticillata. Control animals were given 100 % commercial poultry feed. Clinical signs, changes in body weight, and feed consumption were recorded periodically. All chickens were euthanatized at the end of the seven-day feeding period.

Dosing Studies. Chickens were divided into 8 groups consisting of up to ten animals each. The chickens were administered 1 % body weight of dried Asclepias daily in divided doses. The plant was administered in gelatin capsules (#000) containing approximately 0.9 grams of ground leaf and stem material. Clinical signs, changes in body weight, and feed consumption were recorded periodically during the seven-day period.

Postmortem Examinations

Complete necropsies and histopathological examinations were performed on sheep and chickens that died or were euthanitized. Tissue

samples were taken from the myocardial wall, lung, liver, kidney, intestinal tract, and pancreas, and were fixed in 10 % formalin. Tissues were processed by a Lipshaw Trimatic automatic tissue processor* and cut approximately 0.1 microns thick using a Model 1512 Leitze microtome**. Slides were stained with hematoxylin & eosin***(H&E).

*(Model 1512, Ernst Leitz Wetzlar, Midland, Ontario)

** (Harleco, Gibbstown, NJ using a Histomatic slide stainer)

*** (Fisher Scientific, Pittsburg, Pa.).

CHAPTER IV

RESULTS

Experiments in Sheep

Clinical Observations. During the first 24 hours after exposure to Asclepias subverticillata, the sheep frequently urinated and at various times displayed frequent head shaking, hyperesthesia, anorexia, mouth licking and lip twitching. Episodes of intermittent convulsions lasting 30 to 45 minutes, began approximately 32 hours after exposure. During the convulsive periods, the sheep was comatose, panting, and lying in lateral recumbency with extensor rigidity. The condition of the sheep significantly improved after the convulsive period and was capable of standing and walking. About 40 hours after initial exposure, the sheep became moribund, and death occurred within 48 hours after exposure.

Asclepias verticillata was given at 0.2% body weight on two consecutive days. Head pressing, lip twitching, and hyperesthesia were observed between 32 - 48 hours after exposure. The supply of plant was insufficient to continue dosing and the experiment was terminated.

A second sheep was given two doses of A. verticillata at 0.4 % body weight at a later time after additional plant had been collected. Eight hours later, frequent urination, marked anorexia, and hyperesthesia were noted. During the first 24 hours after exposure,

fine muscle tremors and twitches of the upper and lower lips were noted. Convulsive seizures, stiff extensor rigidity, opisthotonos, and star gazing were observed between 24 and 30 hours post exposure. During the convulsive stage, the sheep was in lateral recumbency, insensitive to painful stimuli and constantly banged its head against the wall. The respiration rate, rectal temperature and heart rate increased during the convulsive episodes. The sheep was euthanized to prevent further self-mutilation.

Administration of Asclepias incarnata resulted in head shaking, pruritis, irritability, alertness, head butting, and head pressing. A moderate dyspnea rapidly progressed to a severe, constant respiratory groan that was accompanied by shallow rapid respiration. There was no evidence of depression or anorexia. All clinical signs subsided within 30 hours after the last dose was administered.

Similar signs were observed in sheep that were exposed to A. viridis, A. latifolia, A. labriformis, and A. hirtella. Signs of intoxication included moderate to severe anorexia, pallor, diarrhea, weakness, dyspnea and depression. Respiration became slow and deep abdominal in nature but later became shallow and rapid prior to death. Sheep given A. viridis and A. labriformis had very distinct sour odors of the entire body. An obvious ocular flare was present in the right eye of the sheep treated with Asclepias latifolia. In addition, the sheep treated with A. viridis had a watery bile stained diarrhea, muscle weakness, and muscle tremors. Approximately 72 hours after exposure the sheep was reluctant to ambulate, became cyanotic, and collapsed 24 hours prior to death. Asclepias hirtella was associated with a long quiet prodromal period of approximately 32 hours, after which, severe dyspnea,

moderate bloat, and rear limb ataxia developed rapidly. Death occurred within four hours of the onset of dyspnea.

Signs of uneasiness, irritability, eye and lip twitching, head pressing, head shaking, head butting, constant eating, constant walking, constant alertness, colic and bloat without diarrhea were associated with Asclepias syriaca.

Few signs were observed in sheep given Asclepias speciosa and Asclepias tuberosa other than a mucopurulent nasal and ocular discharge, and slightly loose fecal pellets.

Electrocardiography. Increased heart rates occurred during the first 24 hours after sheep were exposed to A. subverticillata, A. labriformis, A. verticillata (0.4 % bwt), A. hirtella, A. speciosa, A. incarnata, and A. tuberosa in contrast to decreased rates in those sheep exposed to A. viridis, A. latifolia, and A. syriaca. Changes in amplitude and duration of intervals were noted in some species of Asclepias. The amplitude of the P wave increased with A. syriaca and A. verticillata(0.4% bwt); however, with A. syriaca, the R wave amplitude decreased in contrast to the increase seen with A. verticillata. Asclepias latifolia was associated with a prolonged PR interval and a decreased heart rate. Late to terminal ventricular fibrillations were evident in sheep given A. subverticillata, A. hirtella, A. labriformis and A. viridis.

Clinical Pathology. Creatine phosphokinase(CPK) concentrations were higher than preexposure baseline values in sheep exposed to A. labriformis, A. verticillata(0.2% bwt), A. subverticillata and A. viridis. The CPK values of A. syriaca, A. verticillata, A. tuberosa, and A. hirtella treated sheep increased after exposure but remained in the normal range.

The concentrations of lactate dehydrogenase(LDH) increased in sheep given A. labriformis, A. subverticillata, A. latifolia, A. syriaca and A. verticillata, however, the values did not exceed the accepted reference range for normal adult sheep.

Fibrin values increased above preexposure baseline levels but remained within the normal range in sheep dosed with A. syriaca, A. incarnata, A. speciosa, A. labriformis, A. subverticillata, however, Asclepias viridis was associated with levels that exceeded the upper normal range.

Serum phosphorus decreased below the normal reference range in sheep treated with A. tuberosa and A. speciosa. The decreased phosphorus levels that occurred in A. latifolia and A. hirtella treated sheep remained within normal range.

Total white blood cell counts(WBC) increased in sheep dosed with A. syriaca, A. tuberosa, and A. incarnata. Mean corpuscle hemoglobin concentration(MCHC) decreased in A. speciosa and A. verticillata exposed sheep. Asclepias tuberosa was associated with a decreased mean corpuscular hemoglobin level. Both MCHC and MCH decreased in sheep treated with A. labriformis and A. viridis.

Gross Pathology. Necropsies were not conducted in sheep treated with A. tuberosa, A. verticillata(0.2 %), A. incarnata, and A. speciosa since these sheep survived the experiments. Sheep treated with A. subverticillata, A. labriformis and A. hirtella had congested and edematous lungs, hemorrhage in the pancreas, and subserosal hemorrhage in the pyloric and duodenal areas. Other lesions that were observed in the sheep given A. hirtella included pale whitish foci and petechial hemorrhages in the liver, tracheal edema, moderately increased peri-

cardial fluid, fine visible white streaks in the myocardium and telangiectasis in the lungs. The sheep given A. labriformis had focal areas of pallor in the myocardium and the distal trachea and proximal bronchi were filled with frothy fluid. The A. latifolia exposed sheep had pulmonary emphysema, caseous lymphadenitis. In addition, there was a granular appearance of the liver, and hemorrhage and excessive fluid in the trachea.

Histopathology. There was mild to severe pulmonary congestion and edema (Figure 2) in all sheep that died as indicated in Table III. In addition, alveolar and bronchiolar congestion and edema were seen in sheep treated with A. hirtella, A. verticillata (0.4 %), and A. latifolia. Moderate pulmonary hemorrhage was evident in A. viridis exposed sheep.

Mild to moderate areas of multifocal cardiomyopathy and mild nonsuppurative myocarditis were seen in A. subverticillata treated sheep (Figure 3). Asclepias hirtella, A. latifolia and A. verticillata were associated with mild nonsuppurative, mononuclear infiltrations in the myocardial interstitium of treated sheep.

There were no significant hepatic lesions in any of the sheep except for mild hepatocyte degeneration and moderate multifocal portal fibrosis with heavy infiltrates of lymphocytes around ectatic bile ducts of the sheep dosed with A. verticillata. Subserosal hemorrhage and hematomas were observed in the duodenum, pancreatic area, jejunum, ileum, and colon of sheep treated with A. labriformis, A. latifolia, and A. subverticillata.

There were no other significant lesions observed in the tissues examined.

TABLE IV

HISTOPATHOLOGICAL OBSERVATIONS IN SHEEP GIVEN ASCLEPIAS

<u>Asclepias</u> <u>species</u>	Heart	Lung	Other organs
<u>A. viridis</u>	NSL*	edema(3) ⁺	NSL
<u>A. labriformis</u>	NSL	edema(2)	GIT:H*
<u>A. hirtella</u>	Infil(1)**	edema(2 ⁺)	GIT:H
<u>A. latifolia</u>	Infil(1)	edema(2 ⁺)	NSL
<u>A. syriaca</u>	NSL	edema(2)	NSL
<u>A. verticillata</u>	Infil(1)	edema(2)	Liver:P*
<u>A. subverticillata</u>	Infil(1) myocarditis degeneration	edema(3)	GIT:H

*Abbreviations

NSL = no significant lesions. Infil=infiltrations. P=portal

H = hemorrhage. GIT= gastrointestinal tract

+Ranking:1=mild,2=moderate,2⁺=moderate to severe, 3=severe

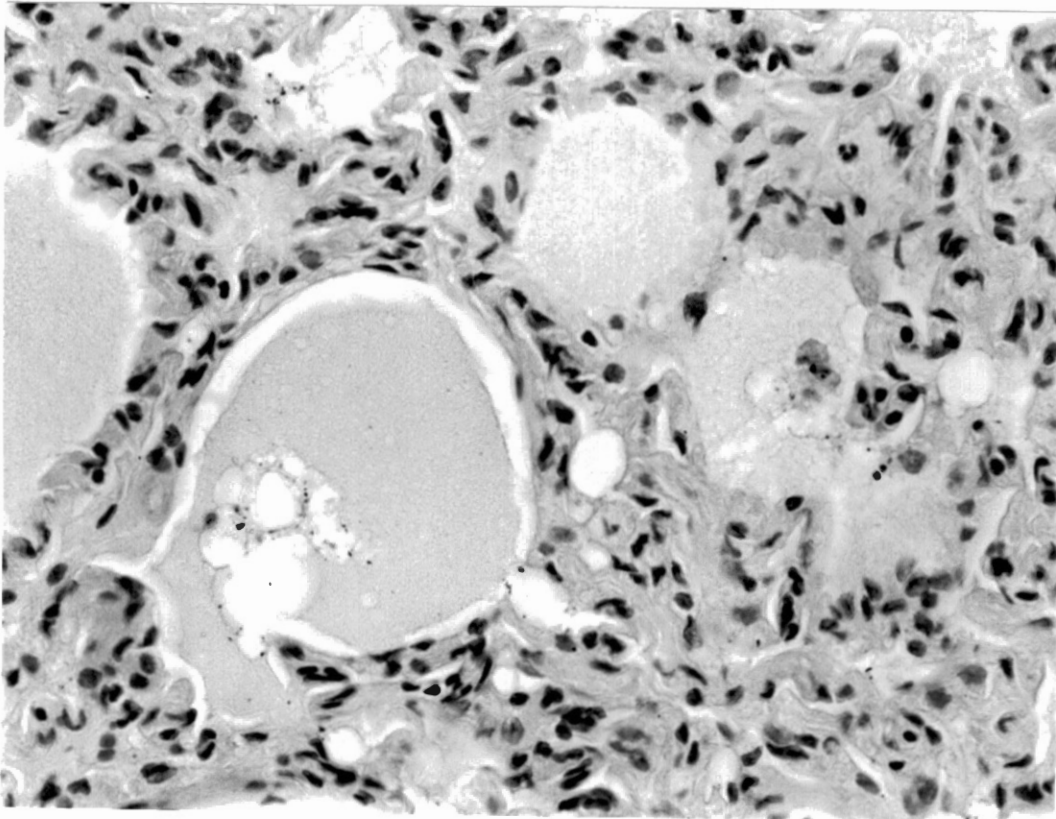


Figure 2. Pulmonary Edema in Sheep given Oral Asclepias

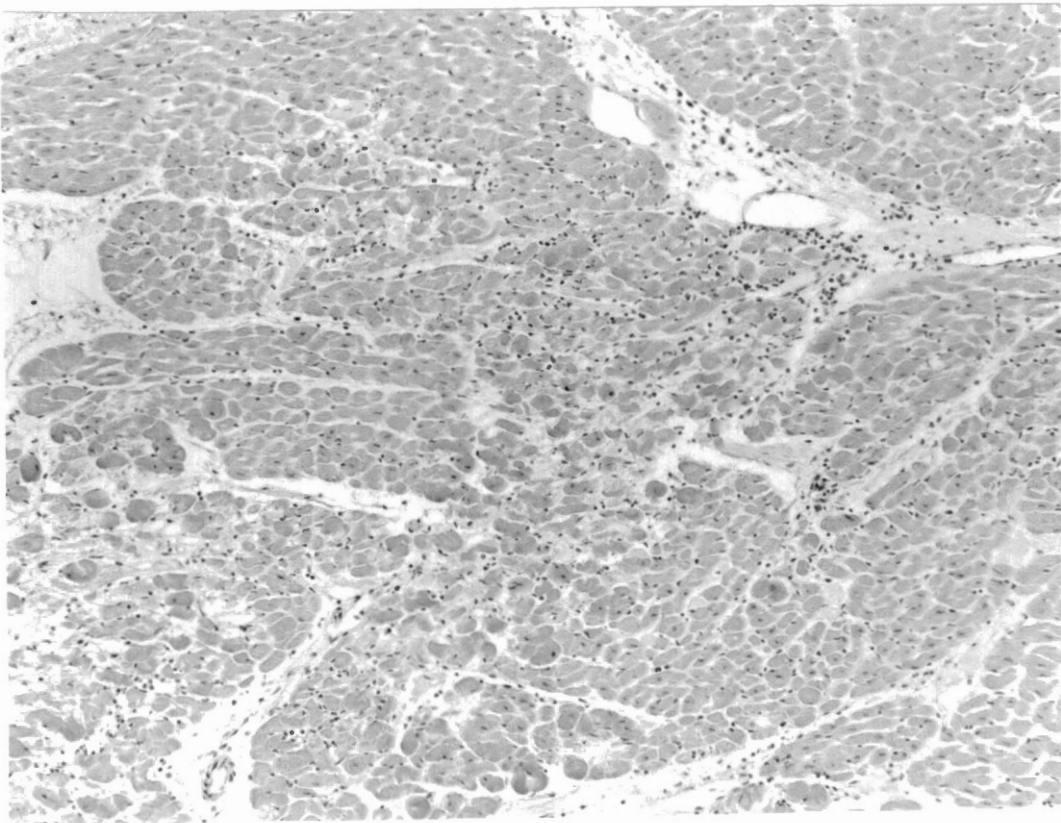


Figure 3. Myocardial Degeneration; Infiltrations of Macrophages and Lymphocytes in the Myocardium of Sheep given A. subverticillata

TABLE V
SUMMARY OF DOSE AND EFFECT OF ASCLEPIAS IN SHEEP

<u>Asclepias</u> species	Dose (% bwt)*	Weight (Kg)	Number of doses	Total Dosage (%bwt)	Effect
A. <u>viridis</u>	.2	74	5	1.0	Death
A. <u>syriaca</u>	.5	66	2	1.0	Death
A. <u>speciosa</u>	.5	50	3	1.5	Normal
A. <u>tuberosa</u>	.5	55	3	1.5	Normal
A. <u>labriformis</u>	.1	51	2	0.2	Death
A. <u>latifolia</u>	.2	66	2	0.4	Death
A. <u>incarnata</u>	.5	53	2.5	1.2	DYS/NS ⁺
A. <u>hirtella</u>	.5	53	2	1.0	Death
A. <u>verticillata</u>	.2	50	2	0.4	NS ⁺
A. <u>verticillata</u>	.4	51	2	0.8	NS
A. <u>subverticillata</u>	.2	53	2	0.4	Death

* dry weight basis

+ Abbreviations

⁺ NS = nervous system signs

⁺ DYS/NS = dyspnea and nervous system signs

Experiments in Chickens, Feeding

Clinical Observations. Asclepias viridis and Asclepias labriformis treated chickens showed signs of anorexia, depression, diarrhea and drowsiness. Respiration was slow and deep abdominal in nature. Several chickens appeared drowsy with their eyes closed and/or their heads tucked underneath the wings. They also refused to eat and subsequently loss weight. All chickens dosed with A. viridis and A. labriformis lived until euthanatized.

Four chickens showed signs of intoxication and died within 72 hours after exposure to A. verticillata. Feeding A. subverticillata resulted in four deaths in less than 24 hours and another chicken died within 72 hours after exposure. Affected chickens showed signs of irritability, hyperesthesia, incoordination, ataxia, loose stools, seizures and death. Survivors of the highly fatal excitatory phase slowly recovered during the depressive stage. During recovery chickens had torticollis, anorexia, weakness, and mild to moderate head tremors.

Chickens refused to eat the A. speciosa and A. syriaca/feed mixtures for 48 and 72 hours, respectfully. Once chickens commenced eating the mixtures, they had increased appetites and voluminous stools. In spite of their increased appetites, there was severe weight loss. One chicken that was exposed to the A. syriaca/feed mixture had engorged corneal blood vessels on the seventh day of the study. Chickens exposed to the A. syriaca constantly stood over the feed containers. No deaths occurred in the chickens given A. speciosa or A. syriaca.

Chickens fed A. tuberosa exhibited anorexia, weight loss, depression, diarrhea and 3 of the 4 had engorged corneal blood vessels.

There were no abnormal signs detected among control chickens.

Feed Consumption and Body Weight Change. All the chickens given the 50 % Asclepias/feed mixture consumed less total feed and lost weight compared to feed consumption and weight gain in the control chickens as shown in Table VI.

TABLE VI

MEAN CONSUMPTION, BODY WEIGHT CHANGE, MORTALITY OF CHICKENS FED
ASCLEPIAS

<u>Asclepias</u> species	<u>Asclepias</u> consumed (g)	Commercial feed consumed(g)	Body weight change(g)	Mortality %	N***
<u>A. viridis</u>	15	15	-656*	0	4
<u>A. syriaca</u>	35	35	-300*	0	4
<u>A. speciosa</u>	45.5	45.5	-294**	0	4
<u>A. tuberosa</u>	5	5	-428*	0	4
<u>A. labriiformis</u>	6	6	-360*	0	4
<u>A. verticillata</u>	3.5	3.5	-386*	50	8
<u>A. subverticillata</u>	5.5	5.5	-211*	62.5	8
Control	0	992	+117*	0	4

* significant at $p < 0.01$.

** significant at $p < 0.05$.

*** Abbreviations

N=number of chickens used.

Gross Pathology. There were no visible lesions in either experimental or control chickens with one exception. Chickens given the A. verticillata and A. subverticillata / feed mixtures had several bruises of the skin and musculature and hematomas subsequent to trauma.

Histopathology. Mild pulmonary edema was seen in chickens fed A. viridis, A. labriformis, and A. subverticillata. In addition, mild to moderate infiltrates consisting of heterophils and macrophages were observed in the myocardial interstitium of chickens exposed to A. verticillata and A. subverticillata.

Experiments in Chickens, dosing

Clinical Observations. Chickens dosed with A. viridis and A. labriformis showed signs of anorexia, depression, drowsiness, stasis of the proventriculus, sour body odor, regurgitation, coldness of the extremities, reluctant to move, and cyanosis of the comb and wattles.

Chickens given A. syriaca had increased appetite and voluminous stools throughout the study. They remained alert with empty crops and stood, constantly pecking at empty containers, cage wire and neighboring chickens. Feces were slightly loose but quite voluminous and orange in color. Chickens given Asclepias speciosa were slightly anorexic during the first 24 hours after exposure, but rapidly regained appetite and feces remained slightly loose and dark green. These signs progressively diminished throughout the study. Feces of chickens that consumed A. syriaca became firmer and extremely voluminous while feces of chickens exposed to A. speciosa remained slightly loose and dark green. All chickens remained alert and standing.

Chickens given A. tuberosa showed signs of depression, drowsiness,

anorexia, diarrhea, regurgitation and weakness.

All chickens dosed with A. verticillata and A. subverticillata showed signs of intoxication within eight hours of administration of stuffed gelatin capsules. Asclepias subverticillata and A. verticillata produced signs which could be categorized into initial excitatory and a late depressive stage 24 hours after dosing. During the prodromal period, an explosive diarrhea occurred approximately 2.5 hours after dosing. The excitatory stage was characterized by a sudden onset of convulsive seizures approximately 6.5-8 hours after exposure. There were intermittent to constant violent convulsions, which were accompanied by complete backward flips, resulting in bruises and hematomas in the musculature, wings, and digits. Many chickens had terminal dyspnea often with an expiratory grunt. The excitatory stage was accompanied by increasing depression by 12-16 hours after dosing, leading to the extreme depression approximately 24 hours after initial exposure.

This depressive stage was associated with abnormal posture, anorexia, and hypoesthesia. Upon stimulation and handling there were seizures and intentional tremors. In addition, there were various degrees of torticollis, weakness, incoordination, and inability to stand or walk. The severity of depression and other signs gradually decreased. Within 48 to 72 hours after exposure, abnormal postures such as head hurdling, 90 to 180 degree torticollis, tail wagging, beak balancing and cage pressing were evident. Between 72 to 96 hours post exposure, affected chickens continued to improve, but remained depressed, weak, and incoordinated. In addition, they walked backward only or were unable to walk forward. Most appeared to be slightly more sensitive to

painful stimuli. Evidence of increased environmental awareness, moderate to slight lameness, decreased torticollis and ataxia, increased sensitivity to pain, improved posture, and dysphagia were noted between 120 and 168 hours after exposure. Most affected chickens did not survive the excitatory stage and only one chicken survived both excitatory and depressive stages. Of 20 chickens given 8.8 g of Asclepias verticillata and Asclepias subverticillata, death occurred less than 8 hours after dosing. Mortality rates among the chickens given A. verticillata were as follows: 6 died in less than eight hours, seven chickens died within 24 hours, and eight had died at 72 hours after exposure. Two chickens lived until euthanatized. Among chickens given A. subverticillata, eight died 24 hours after initial exposure, and within 120 hours after dosing, nine chickens were dead. One chicken was barely alive when euthanatized at the end of the study.

Feed Consumption and Body Weight Change. All chickens dosed with Asclepias consumed less feed than controls except those given A. syriaca. All chickens had a net weight loss except those given A. speciosa. A total of approximately 135 grams of A. viridis, A. syriaca and A. tuberosa were given to chickens during the seven-day period. The greatest net weight loss occurred with A. syriaca and A. viridis. Due to deaths and/or severity of clinical illness, chickens treated with A. subverticillata, A. verticillata, and A. labriformis were not administered the originally proposed total dose as in Table VII.

TABLE VII
 MEAN CONSUMPTION, BODY WEIGHT CHANGE, MORTALITY OF CHICKENS
 DOSED WITH ASCLEPIAS

<u>Asclepias</u> species	<u>Asclepias</u> consumed (g)	Commercial feed (g)	Body weight change(g)	Mortality %	N***
<u>A. viridis</u>	135	12	-454*	0	4
<u>A. syriaca</u>	135	1350	-290*	0	4
<u>A. speciosa</u>	135	678	+94**	0	4
<u>A. tuberosa</u>	29	68	-201*	0	4
<u>A. labriformis</u>	53	68	-374*	25	4
<u>A. verticillata</u>	15	0	-190*	90	10
<u>A. subverticillata</u>	13	0	-190*	90	10
Control	0	989	+109*	0	4

* Significant at $p < 0.01$.

** Significant at $p < 0.05$.

*** Abbreviations.

N = number of chickens used.

Gross Pathology. Chickens dosed with A. viridis and A. labriformis had a sour body odor, cyanotic combs and wattles, and plaques of indurated and ulcerated epithelium in the proventriculus. Hyperemia and scattered foci of hemorrhage were seen in the duodenum, jejunum, spleen and pancreas of a few of these chickens given.

Except for scattered, light diffuse areas of granularity in the liver, there were no significant lesions observed in chickens given A. speciosa and A. syriaca. Two chickens given Asclepias tuberosa had abdominal air sacculitis and one had peritonitis.

There were numerous bruises and lacerations of the skin, pectoral muscles, cervical area, and skin, loss of feathers, denuded skin and hematomas on the wings of chickens of chickens given A. verticillata and A. subverticillata. Other observations included congestion, hyperemia and scattered petechial hemorrhages in the intestinal tract. There were no lesions in chickens that did not show signs of illness.

Histopathology. There were no lesions observed in the heart, lungs, liver, kidney or intestinal tract of control chickens. Congestion and edema were observed in the lungs of chickens dosed with A. viridis, A. tuberosa, A. syriaca and A. speciosa. Mild to severe infiltrations of heterophils and macrophages were observed in the myocardium of chickens intoxicated with A. verticillata, A. subverticillata, and A. labriformis. All chickens that died within eight hours of dosing had lesions in the myocardium (Figure 4). Histopathological ranking of myocardial lesions from greatest to least severity of lesions: A. verticillata = A. subverticillata > A. labriformis > A. viridis = A.

syrica > A. speciosa = A. tuberosa = controls.

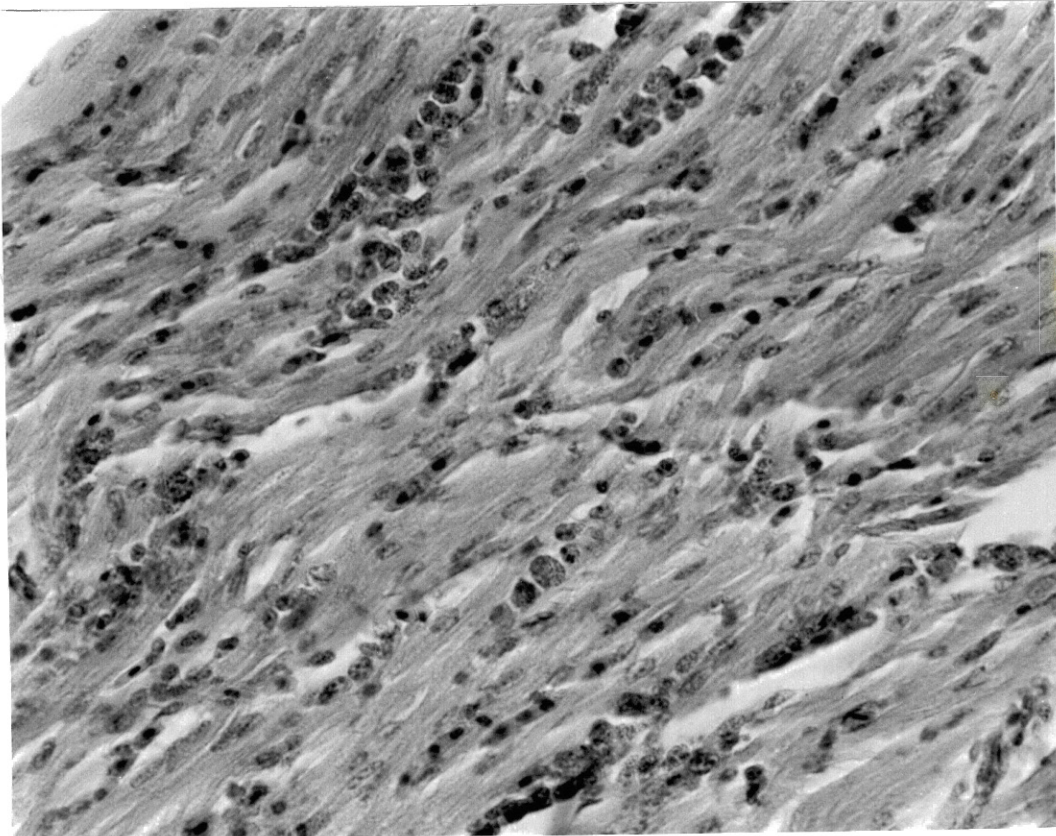


Figure 4. Infiltrations of Macrophages and Heterophils in the Myocardial Interstitium of Chickens given A. verticillata

CHAPTER V

DISCUSSION

These studies were initially carried out in a limited number of chickens fed a mixture of Asclepias and commercial feed. Because of failure to consume sufficient feed to cause intoxication, these studies were extended to similar numbers of chickens given Asclepias by direct dosing in gelatin capsules. Subsequently sheep were administered the plants but in most instances one sheep per species of plant was used. This allowed for limited comparison of the results between birds and mammals. It also gave a more useful comparison of toxicity between Asclepias species. The use of large numbers of sheep was not possible in conjunction with the numerous Asclepias species used.

Previous reports have attributed the toxicity of Asclepias mainly to their cardiac glycoside content (Seiber et al., 1979, 1983). Clinical signs and lesions observed in sheep and chickens given A. viridis, A. labriformis, A. latifolia, A. hirtella were consistent with cardiac glycoside intoxication (Amory et al., 1905; Gaze et al., 1961; Benson et al, 1979; Merck, 1982). In the present studies the clinical signs which included depression, anorexia, diarrhea, and dyspnea in addition to pulmonary edema observed in sheep and chickens were consistent. However, the heart rates and electrocardiographical changes were not consistent. Cardiac glycosides affect $\text{Na}^+ - \text{K}^+$ ATPase, and are thought to alter normal contractility of the myocardial fibers

or directly damage myocardial fibers leading to cardiac insufficiency. If the insufficiency is prolonged, there is usually some compensation by cardiac dilation and hypertrophy. In the present studies there was no pathological evidence of heart failure in any of the sheep, however, the increased heart rates could have been a physiologic systemic response to cardiac insufficiency.

The histopathological findings revealed foci of myocardial degeneration which indicated that myocardial damage was present in the sheep. The inability of the heart to adequately pump blood resulting in a vascular pressure increase in the alveoli may have caused the dyspnea and pulmonary edema present in these sheep. However, the myocardial lesions were not as severe as expected, perhaps related to the short duration of exposure and acute deaths. It seems unlikely that the mild myocardial lesions could account for the severity of pulmonary edema. This may be suggestive of a direct vascular effect of the toxicant and or a multiple effect.

Chickens exposed to A. verticillata, A. subverticillata and A. labriformis had both heterophils and macrophages in the myocardium, suggestive of inflammation secondary to myocardial damage.

The pulmonary lesions and dyspnea that occurred in sheep were similar to findings reported by Benson(1979), however, there were no significant lesions observed in the kidney (Benson et al., 1979) or liver(Pahwa et al., 1988).

The presence of myocardial and pulmonary lesions in chickens and sheep that exhibited a neurological syndrome are indicative of multiple toxicants at least for some species of Asclepias since it seems unlikely that there is a connection between these diverse effects. Signs

recorded in this study indicate that there were at least two clinical manifestations of Asclepias intoxication that should be recognized. The neurotoxic species, A. verticillata and A. subverticillata were associated with irritability, convulsions, and opisthotonus without dyspnea or diarrhea which were so characteristic of the other Asclepias species. While increased heart and respiration rates were apparent in all sheep which showed signs of intoxication signs, a moderate to severe dyspnea and diarrhea were limited to sheep treated with A. labriformis, A. hirtella, A. latifolia, and A. syriaca. This finding indicates the presence of multiple toxicants in Asclepias species, which should be considered prior to treatment.

Previous reports(Marsh,1920,1924) indicate that A.verticillata is of limited toxicity and only about 1/10 as toxic as A. subverticillata, however, this study showed there was less of a differential in toxicity. Perhaps there were differences in subspecies of the plants and/or geographical differences, climatic and growth seasons and sampling which might account for differences in the toxicities. The approximate ratio of lethal/toxic dose of A. subverticillata compared to A. verticillata in chickens was 1:1.2 and approximately 1:4 in sheep which markedly differs from the reported 10 fold differential toxicity.

Due to their prevalence in Oklahoma, some less well studied species such as A. viridis, A. syriaca, and A. speciosa could be potential hazards to livestock. A greater potential hazard may exist after exposure to A. speciosa because sheep and chickens consumed this species more readily than others used in the study.

A. syriaca was associated with increased appetite or feed consumption in general. While A. viridis was associated with both marked feed refusal

and severe anorexia, its extreme abundance increases the potential for exposure. Based on the results of this study, these species may likely be associated with anorexia, decreased production and efficiency perhaps instead of a sudden death syndrome. This could be confusing to the clinician and owner. Although it was not lethal in the present experiment, A. incarnata caused severe dyspnea and significant nervous system signs and may be expected to be lethal at total doses slightly greater than 1.2% or if it were eaten over a longer period. The clinical signs appeared to involve both nervous and gastrointestinal /cardiovascular systems which supports the hypothesis (Woodson, 1954) that A. incarnata is a further gradation of A. verticillata encountered as one traverses from the midwestern to the eastern U.S. Both analytical and toxicological studies should be conducted to substantiate this theory.

Asclepias tuberosa and A. speciosa caused few clinical effects in sheep at 1.5% body weight however, both caused anorexia and diarrhea in chickens. The lack of effect in sheep may have been due to insufficient dosage.

Because of the many differences between the Asclepias species, it is difficult to assess the degree of toxicity on the basis of clinicopathological or electrocardiographic findings. The ECG appears to be of limited use as a diagnostic tool because few significant changes were apparent and some such as ventricular fibrillation, occurred only terminally when death was eminent. More information regarding normal electrocardiograms and clinicopathological values in sheep are needed to utilize such parameters in making a prognosis.

Clinical observations such as respiration rate and character of respiration serve as better indicators of severity of toxicity in sheep. This study did not confirm the depressed and/ or fluctuations in body temperature as cited in earlier reports(Marsh et al., 1921,1924).

A relative comparison of dose and effect as shown in Table IV indicated that Asclepias labriformis was the most toxic while Asclepias tuberosa and Asclepias speciosa were the least toxic of the milkweeds studied. The lethal dose of Asclepias subverticillata and Asclepias latifolia was half that of Asclepias viridis and Asclepias incarnata. The lethal dose of Asclepias syriaca was twice that of Asclepias subverticillata and Asclepias latifolia. Asclepias incarnata and Asclepias verticillata did not cause death, but both caused severe signs of intoxication. Asclepias tuberosa and Asclepias speciosa did not cause significant clinical signs of intoxication at a dose of more than seven times the lethal dose of Asclepias labriformis.

CHAPTER VI

SUMMARY

This study evaluated the effects of several species of Asclepias in 6 week old male chickens and in adult cross bred white faced female sheep. Dried ground plant material was administered daily up to 7 days in chickens and 5 days in sheep. Based on clinical signs there appeared to be at least two clinical syndromes associated with Asclepias intoxication.

A. subverticillata and A. verticillata caused a neurological syndrome in sheep characterized by irritability, seizures, convulsions and death. Chickens showed an excitatory and a depressive phase after exposure to these neurotoxic species. Sheep dosed with A. viridis, A. labriformis, A. latifolia, A. syriaca and A. hirtella showed signs of progressively moderate to severe dyspnea, diarrhea, anorexia, depression, and death occurred. Asclepias incarnata was associated with both neurological signs and gastrointestinal signs, however, exhaustion of plant supply terminated study of the plant.

Clinical signs observed in chickens dosed with A. labriformis and A. viridis were similar to those seen in sheep. There were few clinical effects observed in sheep treated with A. speciosa and A. tuberosa.

There were few gross pathological lesions were observed on necropsy of these animals. Histopathological examination of tissues

revealed varying degrees of pulmonary congestion and edema in sheep that died. Macrophage and heterophic infiltrates were observed in the myocardial interstitium of chickens given the A. verticillata , A. subverticillata and A. labriformis.

Electrocardiographic and clinicopathologic parameters were of limited assistance in determining mechanisms and pathogenesis. Dyspnea, diarrhea, and respiration rates would be useful criteria in monitoring toxicity.

BIBLIOGRAPHY

- Amory, R. and Emerson, R. (1905) Wharton and Stille's Medical Jurisprudence vol II Poisons (5th ed), 510-520, 772-779). Rochester: The Lawyers' Co-Operative Publishing Company.
- Benson, J., Seiber, J., Bagley, C., Keeler, R., Johnson, A. and Young, S. (1979) Effects on Sheep of the Milkweeds A. eriocarpa and A. labriformis and of Cardiac glycoside-containing Derivative Material. *Toxicol.* 17, 155-165.
- Benson, J., Seiber, J., Keeler, R., and Johnson, A. (1978) Studies on The Toxic Principle Of Asclepias eriocarpa and Asclepias labriformis. In: Effects of Poisonous Plants on Livestock. Keeler, R. (ed) p. 273-284) San Francisco: Academic Press.
- Brower, L. (1968) Ecological Chemistry and the Palatability Spectrum. *Science*. 161, 1349 -1350.
- Brower, L. (1969) Pierson. Ecological Chemistry. *Scientific American*. 220, 22 -29.
- Brower, L. (1974) Palatability dynamics of cardenolides in the Monarch butterfly. *Nature*. 249, 280-282.
- Brower, L., Gibson, D., Moffitt, G., and Panchen, A. (1978) Cardenolide content of Danus Chrysippus butterflies from three areas of East Africa. *Biological J. of Linnean Society*. 10, 251- 273.
- Brower, L., Mc Evoy, P., Williamson, K., and Flannery, M. (1978) Variation in Cardiac Glycoside content of Monarch Butterflies from natural Populations in Eastern North America. *Science*. 177, 426-428.
- Campbell, H. (1931) Poisoning in Chickens with Whorled Milkweed. *Journal Of American Veterinary Medical Association*. 79, 102 - 104.
- Clark, J. (1979) Whorled Milkweed Poisoning. *Vet & Hum Toxicol*. 431.
- Couch, J. (1929) Experiments with extracts from the wooly-pod milkweed (Asclepias eriocarpa). *Am. J. Pharmacy*. 101, 815.
- Edwards, W. Burrows, G. and Tyrl, R. (1984) Toxic Plants of Oklahoma-- Milkweeds. *Journal of Oklahoma Veterinary Medical Association* 36, 74-79.
- Ehrlich, P., and Raven, P. (1967) Butterflies and Plants. *Scientific American*, 35.

Emon, J. and Seiber, J. (1985) Chemical constituents and Energy Content of Two Milkweeds, Asclepias speciosa and Asclepias currasavica. Economic Botany. 39, 47 - 55.

Enson, J. and Seiber, J. (1978) High-speed liquid chromatography of cardiac glycosides in milkweed plants and monarch butterflies. Journal of Chromatography. 148, 521-527.

Euw, J., Fishelson, L., Parsons, J., Reichstein, T., and Rothschild, M. (1967) Cardenolides (Heart Poisons) in a Grasshopper Feeding on Milkweeds. Nature. 214.

Gabor, S. and Agnes, P. (1987) A szarvarmarhak selyemkoro-mergezese (Asclepias syriaca). Magyar, Allatorvosok Lapja. 42(1), 56-58.

Gazes, P., Holmes, C., Moseley, V., and Rawling, H. (1961) Acute Hemorrhage and Necrosis of the Intestines Associated with Digitalization Circulation. 23:358-364.

Glover, G. (1917) The Whorled Milkweed. American Journal of Veterinary Medicine. 12, 303-338.

Glover, G., Newsom, I. and Robbins, W. (1918) A new Poisonous Plant. Colorado Agriculture Experiment Station Bulletin. 246.

Glover, G., and Robbins, W. (1915) Whole Milkweed (Asclepias verticillata). In Colorado Plants Injurious to Livestock, Agriculture Experiment Station, Col Agriculture College, Bulletin. 211, 60.

Isman, M. (1977) Dietary Influence of Cardenolides on Larval Growth And Development of the Milkweed Bug, Oncopeltus Fasciatus. Journal Insect Physiology. 23, 1183.

Kingsbury, J. (1964). Poisonous Plants of the US and Canada. (pp. 267-269). Englewood, NJ: Prentice Hall Inc.

Kupchan, S. Knox, J., Kelsey, J. and Renauld, J. (1964) Calotropin, a Cytotoxic Principle Isolated from Asclepias curassavica L. Science. 1686-1687.

Mahmoud, O., Adam, S., and Tartour, G. (1979) The Effects of Calotropis Procera on Small Ruminants. I. Effects of Feeding Sheep with The Plant. Journal of Comparative Pathology. 89, 241-250.

Mahmound, Adam, S., and Tartour, G. (1979) The Effects of Calotropis Procera On Small Ruminants. II Effects of Administration of the Latex to Sheep and Goats. Journal of Comparative Pathology. 89, 251-255.

Marsh, C., Clawson, A., Couch, J., and Eggleston, W. (1920) The Whorled Milkweed (Asclepias galioides) As A Poisonous Plant. USDA Bulletin # 800.1-40.

Marsh, C., and Clawson, A. (1921) The Mexican Whorled Milkweed (Asclepias mexicana) As A Poisonous Plant. USDA Bulletin # 969, 1-16.

- Marsh, C., and Clawson, A.(1921) The Mexican Whorled Milkweed(Asclepias mexicana)As A Poisonous Plant. USDA Bulletin # 969,1-16.
- Marsh,C.,and Clawson,A.(1921)Poisonous Properties of the Whorled Milkweeds Asclepias pumila and Asclepias verticillata var Geryeri. USDA Bulletin # 942, 1-14.
- Marsh,C.,and Clawson,A.(1924) The Woolly-Pod Milkweed(Asclepias eriocarpa)As A Poisonous Plant. USDA Bulletin # 1212,1-14.
- Merck Manual of Diagnosis and Therapy(1982) Diseases of the Heart and Pericardium, Congestive Failure.Merck Sharp & Dohme Research Laboratories. Merck & Co.,Inc., 422-424.
- Milks,H. and Eichhorn,A.(1943) Practical Veterinary Pharmacology, Materia Medica and Therapeutics.(5th ed),82-94,Chicago:Alexander Edger.
- Pahwa,R.,Chatterjee,V.(1988). The Toxicity of Indian Calotropis porcera RBr Latex in the Black Rat, Rattus Rattus Linn. Veterinary & Human Toxicol,30(4),305-308.
- Pammel,L.(1911) A Manual of Poisonous Plants. Part I,130 Cedar Rapids: The Torch Press.
- Rothschild,M.(1973) Secondary plant substances and warning colouration in insects. Royal Entomological Society of London(Symposium #6)(ed)Van Emden. Blackwell Scientific Publisher, London, p. 59.
- Rowe, L., Ohlenbusch,P. and Dollahite,J.(1970) Toxicity of Asclepias brachystephana(Shortcrown Milkweed) for Sheep. The Southwestern Veterinarian. Spring, 219 - 224.
- Sayre,L.(1899) A Manual of Organic Materia Medica and Pharmacognosy.295-296. Philadelphia:P.Blakiston's Son & Co.
- Seiber, J., Roeske,C.and Benson,J.(1978) Three New Cardenolides from the Milkweeds Asclepias eriocarpa and Asclepias labriformis Phytochemistry. 17, 967 - 970.
- Seiber, J.,Lee, M.and Benson, J.(1983) Cardiac Glycosides (Cardenolides)in Species of Asclepias(Asclepidaceae).In Plant and Fungal Toxins(I,R.Keeler & A.Tu eds).New York:Marcel Dekker.
- Seiber,J.,Lee,S.,McChesny,M., Watson,T. & Nelson,C. and Brower,L.(1985) New Cardiac Glycosides(Cardenolides)From Asclepias Species. In: Plant Toxicology Proceedings of the Australia--USA Poisonous Plants Symposium,Bisbane, Australia.May 14-18, 1984, 427-437,(A.Seawright, M.Hegarty, L.James,R.Keeler, eds). Univeristy of Queensland:Yeerongpilly.
- Smith, D.(1979) The Significance of beak marks on the wings of an aposematic, distasteful and polymorphic butterfly.23.

Sprawls, R (1982) Horsetail Milkweed Intoxications in Horses and Cattle. Southwestern Veterinarian 35, 15.

Stiles, G.(1942) Poisoning of Turkey Poults from Whorled Milkweed (Asclepias galioides). Poultry Science. 21, 263-269.

Tunnickliff, E.and Cory,V.(1930) Broad-Leafed Milkweed(Asclepias latifolia) Poisonous For Sheep and Goats. Journal of American Veterinary Medical Association. 77,165-168.

Vail, E.(1942) Woolly-Pod or Broad-Leafed Milkweed(Asclepias eriocarpa)Poisoning of Rabbits. North American Veterinarian. 23, 539 - 542.

Woodson,R.(1954) The North American Species of Asclepias L. Annals of the Missouri Botanical Garden. 41(1),1-199.

VITA²

Lanell Ogden

Candidate for the Degree of
Master of Science

Thesis: TOXIC EFFECTS OF MILKWEEDS (ASCLEPIAS) IN SHEEP AND CHICKENS

Major Field: Physiological Sciences

Biographical:

Personal Data: Born in Baxley, Georgia, September 19, 1955

Education: Graduated from Appling County High School, Baxley, Georgia, in June, 1973; received Bachelor of Science Degree in Biology at Fort Valley State College, Fort Valley, Georgia in June, 1977; received Doctor of Veterinary Medicine at Tuskegee University, Tuskegee, Alabama, in May, 1981; completed requirements for the Master of Science degree at Oklahoma State University in July, 1989.

Professional Experience: Toxicology Resident, Oklahoma Animal Disease Diagnostic Laboratory, July, 1987, to July, 1989.