THE TOXICITY OF OKLAHOMA SPIDER (ARANEAE) VENOMS USING A NEW VENOM RECOVERY AND TESTING TECHNIQUE

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PREFACE

This research project was suggested by Dr. D. E. Howell, Professor and Head, Department of Entomology, Oklahoma State University. Being a medical entomologist in the United States Navy, I was immediately attracted to this idea because it represented an opportunity to become trained in a field little understood by entomologists in general. More importantly, it represented the opportunity to add another dimension to my ability to provide technical assistance for the Naval Establishment.

Indebtedness is expressed to: The United States Navy for making the study possible; Dr. E. D. Besch, Professor and Head, Department of Veterinary Parasitology and Public Health; and Dr. R. R. Walton, Professor of Entomology for criticisms and suggestions in carrying on the research and in reviewing the manuscript; Dr. Harriet Exline (Mrs. D. L. Frizzell), Rolla, Missouri for identifying the specimens used in the research; Mr. Sidney Kunz, Don Arnold, and Charles Bailey for aiding in the collection of spiders; Janet Doles Grothaus, my wife, for aid in preparing the manuscript.

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INTRODUCTION

Many arthropods possess mechanisms for the injection of venoms which can cause harmful or lethal effects in humans. The spiders in particular have been obscured by a curtain of mystery and superstition for centuries. Only in recent years have investigators, employing modern scientific methods, made appreciable progress in gaining knowledge concerning spiders and their venom.

With the advent of a renewed interest in spiders and the toxicity of their venom, investigators have discovered new, potentially dangerous species of spiders in the United States. The research has been limited to only a few species with each investigator using a different testing procedure. Because of the confusing reports concerning spiders and the relative lack of data, I became interested in attempting to develop a venom recovery and testing procedure that would make possible a more exact comparison of venom toxicity among the many species. The second objective consisted of evaluating as many species of Oklahoma spiders as possible to determine their venom potential. The third objective involved studying the known toxic species with respect to venom dosage and clinical response. The entire study was oriented toward obtaining a better understanding of the public health importance of spiders.

REVIEW OF LITERATURE

The history of knowledge about venoms has been adequately reviewed by Leake (1956) so the review presented in this paper will be limited to material within the scope of the research problem.

Spiders and Their Impact on Public Health

Horen (1966) said that arachnidism statistically was not a serious medical or public health problem. He listed a total of 615 cases of poisonous spider bites up to the early 1940's with 38 deaths recorded. From 1950 through 1959 he reported 65 deaths from spiders. Scott (1963) said that injury from arthropod venoms was a common public health hazard in the United States. He reported that about 25,000 arthropod envenomizations a year result in severe injury with spiders being rated as second in number of human fatalities, bees being the only group causing more deaths. According to him an average of eight people die each year as a result of spider bites.

Known Species of Dangerous Spiders in the United States

Most of the information concerning the spiders that are poisonous to humans has come from clinical situations where the patient has produced or described a spider. One must have serious doubts as to the validity of species named under these circumstances. Harmon and Pollard (1948) present a bibliography of the literature on all aspects of venom and venomous animals. Only the reports which appear to be valid beyond doubt, will be reviewed in this paper.

The most infamous American spider has been the black widow. Herms, Bailey and McIvor (1935) stated that this spider was first described in 1775 as Aranea mactans (Fabricius). It is now known as Latrodectus mactans (Fabr.) according to Fitch (1963). Herms et al. (1935) did some of the early investigation on this species and were able to cause the death of 250-500 gm guinea pigs by causing female black widows to bite them. Horan (1966) reported that the venom was predominantly neurotoxic. He also reported that liver necrosis may occur. D'Amour, Becker and Van Riper (1936) dissected out the poison glands, injected the recovered venom into white rats and determined the LD 50 to be 0.032 mg/kg of body weight. Baerg (1959) described his symptoms after permitting a black widow to bit him: sharp pain was noted at the bite site and aching pain was present in the back. His attending physician noted phagocytosis locally around the bite site. Herms et al. (1935) noted one of the few instances where local necrosis occurred after a bite of this species. They observed that on a guinea pig a two inch area of skin had sloughed from around a one month old bite. They reported that the usual reaction consisted of localized swelling and redness around the bite site. Bogen (1956) stated that necrosis was rare following the bite of this species and pointed out that most necrosis occurred as a result of excessive local treatment or secondary infection. There still appears to be some confusion concerning the taxonomy of the black widow. Levi (1959) in a revision of the genus Latrodectus, recognized three species in the United States, L. mactans, L. curacaviensis (Muller) and L. geometricus C. L. Koch. Fitch (1963) reported that the northern black widow, L. curacaviensis had long been confused with L. mactans. He also stated that the northern species

had a less virulent venom. McCrone (1964) found L. geometricus to be surprisingly toxic.

A second genus of spiders is of known major importance in the United States. The genus Loxosceles contains at least one toxic species in the United States (Atkins, Wingo and Sodeman 1957). Gertsch (1958) in a revision of the genus lists 18 species in North American and the West Indies. L. reclusa Gertsch and Mulaik, L. devia G. and M., L. arizonica G. and M., L. unicolor Keyserling and L. rufescens Dufour are known to occur within the continental limits of the United States.

Atkins et al. (1958) subjected rabbits to direct bites of L. reclusa and found that the site of the injection was red and congested within five hours. A wheal appeared in seven hours and slowly increased in size for 24 hours. During the third day the wheal was stabilized and the edges were thickened. A secondary pocket formed below the bite site. By the fifth day the skin at the bite site was bluish-purple. As time progressed the lesion darkened and became dry. On the tenth day in one test the black area sloughed and continued sloughing until the fifteenth day. Healing began and was completed 35 days after the initial bite. Guinea pigs were also bitten and found to develop similar clinical symptoms. Necropsies were done and the significant changes were found to be limited to the site of the bite. The most obvious changes were hemorrhages in the epidermis, subcutaneous tissue and superficial muscular layer. The capillaries were dilated and filled with red blood cells, some of which were fused to form hyalinized masses. They also reported that in humans a systemic response occasionally occurred, characterized by restlessness and fever. Dillaha et al. (1963) reported that in addition to the necrotic lesion, hemolytic

anemia and thrombocytopenia also occurred. Denny, Dillaha and Morgan (1963) in a study of the systemic effects of L. reclusa venom gave intravenous injections to dogs. Depression of the platelet count began within 6 hours. Jaundice and bleeding manifestations occurred after 24 hours. Evidence of hemolysis was not striking. The authors also studied the hemolytic effects of the venom in vitro against human red blood cells. In some instances, hemolysis reached the 26% level after 48 hours. They felt that secondary hemolytic anemia was due to direct lytic action of the venom on the integrity of the cell and was enzymatic in nature. O'Dell et al. (1967) were able to examine the venom using the Conalco polyacrylamide gel disc electrophoresis technique. Protein bands were demonstrated by staining the gel. Biological activity was studied by injection of the recovered fractions into American cockroaches. They reported that the venom contained 5-7 protein components and that only 2-3 were biologically active. It was reported that levarterenol constituted a major component of the venom (Anonymous 1960). Hemolysin of some sort was also reported as being present. Horan (1966) discussed the venoms of the North and South American species of Loxosceles under the same heading, stating that they had a cytotoxic effect with systemic effects frequently occurring concurrently. He reported that temporary increase in the erythrocyte sedimentation rate took place shortly after the venom of L. laeta (Nicolet) was introduced into rabbits. This was followed by leukopenia and shock. Congestive hemorrhagic lesions occurred in the liver and kidneys. Generalized vasodilatation occurred in all animals.

Hite et al. (1966) discussed the biology of L. reclusa in considerable detail; stating that the eggs hatched in 25 to 39 days with as many

as 158 young emerging from one egg sack. The immatures passed through eight instars. The instars varied from one to four months depending on environmental conditions. The minimum and maximum time required for the life cycle was 266 and 444 days respectively. Males lived 301 to 796 days and females lived 356 to 894 days. The temperature extremes tolerated were 40 F and 110 F.

Hite (1964) reported in the field commonly finding this spider under rocks and protected ledges. Gertsch (1949) described the natural habitat of the brown spider to be under rocks, tree bark and in caves. Hite et al. (1966) reported more spiders from indoor sites than outdoor sites. They were most commonly found in boxes, among papers, in bedrooms, attics, halls and utility rooms with the least number being found in basements.

Shulov (1952) studied the venom of L. rufescens in Israel by causing the spiders to bite white mice. Most of the mice died in the tests. Those that lived showed no external signs of necrotic activity. He stated that the symptoms were clearly neurotoxic, showing a clear influence upon the central nervous system and internal organs. However, he did report finding lesions on the liver, and the spleen was enlarged. James et al. (1961) reported a case history of a child that had obviously been bitten by a Loxosceles spider. They checked the home and found specimens of L. unicolor on the premises. However, no laboratory data were presented to confirm the toxicity of the venom. Micks (1963) in a review of the genus Loxosceles in Texas said L. reclusa, L. devia, L. arizonica and L. unicolor were all found in the State but that the relationship between species and severity of symptoms was not known.

Herms and James (1961) reported that Chiracanthium inclusum Hentz

of the family Clubionidae, has been known to produce a temporary painful localized effect. Baerg (1959) confirmed this statement. Furman and Reeves (1957) reported one case where the spider was removed from the patient and identified by competent taxonomists. Baerg (1959) also reported that C. diversum caused the same type of reaction in the State of Hawaii.

Horan (1963) presented a summary of all potentially dangerous spiders in the United States. In addition to those previously discussed, he listed Lyrosa carolinensis (Walckenaer), L. punctulata Hentz, and the genera Pamphobeteus, Pachylomerus, Steada and Eurypelmas. Most of these are listed as causing slight reactions.

Considerable attention has been given to the status of the tarantula Dugesiella hentzi Girard. Baerg (1958) said that this species caused two rats out of six to die after they were bitten. However, he did not consider the species dangerous to man.

Spider Fauna

Gertsch (1949) stated that 2500 species of spiders were known from the Neartic region. Kaston (1953) recognized 40 families and 500 genera in the United States. Very few studies have been done on a state basis. Kaston (1946) recognized about 600 species from Connecticut. Fitch (1963) listed 192 species from a 350 acre tract in Kansas.

Banks, Newport and Bird (1932) cited 160 species from Oklahoma. Branson (1958, 1959) added 25 species and Harrell (1963, 1965) added 20 additional ones. Branson (1966) increased the known fauna by 27 species and Bailey, Grothaus and Drew (1967) added 37. Thus 269 species have been reported for the State.

Venom System

Savory (1928) stated that in the primitive spiders the venom glands were located in the first joint of the chelicerae, while in the more highly developed spiders they were in the anterior end of the cephalothorax. He proposed that the glands were modified salivary glands that had changed function. Comstock (1948) reported that the venom glands were two in number with each gland discharging venom through a long duct which opened near the tip of the apical segment of the chelicera. He stated that the glands were sac-like with the lumen of the sac serving as a reservoir for venom. Kaston (1953) stated that spiders could control the quantity of venom injected. Gertsch (1949) stated that the families Uloboridae and Heptathelidae did not have functioning venom glands.

Methods of Venom Recovery

Most of the venom toxicity studies on spiders have been accomplished by severing the cephalothorax, macerating it in saline and injecting the extract into test animals, (Keegan, Hadeen and Whittemore 1960). D'Amour, Becker and Van Riper (1935) removed the venom glands and macerated them in saline. Shulov (1952) preferred to use a direct bite method because he felt it reduced variability in test results. Lebez (1954) placed a cotton pad between the fangs of the spiders and caused the animals to bite. He recovered venom in various ways and in comparative tests found that fresh venom was more toxic than either dried venom or venom recovered from macerated poison glands. Shulov and Pener-Saloman (1962) therefore proposed that direct bite testing should be used in spider venom research. They realized that the effect of the venom could not be stated in terms of reaction in relation to

weight of venom, but felt that this could be estimated by comparing the data with other methods. Grothaus and Howell (1967) developed a technique that combined the desirable features of older methods. It consisted of placing spiders in expanded plastic cells, holding absorbent pads between the chelicerae and stimulating them to bite with a low distortion sine wave generator. The technique made it possible for the authors to determine the weight of the fresh venom; fresh venom could be used directly rather than dried to obtain the weight; venom was uncontaminated with hemolymph, digestive fluid or body tissue; the amount of venom recovered approximated that released under field conditions, and the spiders were not harmed in the "milking" process.

Methods of Venom Evaluation

Studies on the mammalian toxicity of spider venom have typically been accomplished with a variety of test animals. Baerg (1958) used white rats for studies on tarantulas. Atkins et al. (1958) used rabbits to test the venom of L. reclusa but later switched to guinea pigs because they were less sensitive. Denny et al. (1964) used dogs to study the same species. Lebez (1954) and Shulov and Pener-Salomon (1962) used white mice in their studies. Baerg (1958) stated that test animals were useful in determining the effects of various venoms but were not always reliable in reflecting the effect on humans.

Stahnke (1965) in a study of venoms and stress found that white rats were more sensitive to rattlesnake or scorpion venom after a temperature stress. He found that whether the change was a decrease or increase was not important; the greater the temperature change, the greater the change in toxicity; so variability resulted within the same species of animal because of variable laboratory conditions.

Some use has been made of insects as test animals. Wiener (1956) used Drosophila to assay the venom and antivenom of the spider Lactrodectus hasseltii Thorell. He found that the venom was eight times more toxic to Drosophila than to white mice and suggested that this was because spider venom is most toxic to the organisms upon which the spider feeds. He indicated that the use of Drosophila as an index animal was desirable because of the large number of tests that could be run with a limited amount of venom. It is of interest to note that he used a rabbit to prepare antivenom which was used to neutralize the venom injected into the Drosophila. This would indicate that the venom components harmful to the insect were destroyed as well as those harmful to the rabbit. Bellini (1965) used Musca domestica Linnaeus to study acquired immunity responses against L. mactans and found that the flies developed immunity after sublethal doses just as mammals develop immune responses. Kamon (1965) used migratory locusts to study the difference in toxicity of whole venom and dialyzed venom from the yellow scorpion. Norment (1965) used crickets in an attempt to determine the hemotoxic effect of L. reclusa venom.

All of the authors that used insects injected the spider venom into the body cavity. The authors using mammals used varying techniques. Keegan et al. (1960) used intraperitoneal injections on white mice and in this way discovered that the venom of L. mactans was more toxic in the late summer and fall than it was in early spring. Horan (1963) proposed that deep injections accounted for the lack of local reactions reported by some investigators. He proposed that venoms have the greatest effect when they are injected into tissue of low vascularity (the dermis), because cytotoxic venoms become diluted and lose their strength if they are injected into more vascular tissue.

MATERIALS AND METHODS

Test Organisms

Three species of test animals were used to study the venoms recovered during the period of this research program. White mice were used to determine the mammalian reactions and American cockroaches (Periplaneta americana [Linnaeus]) were used to gain additional information concerning the venom potential of spiders as it related to arthropods. Domestic rabbits (New Zealand whites) were used in one experiment.

The mice used in the tests were a special strain (CD-1) reared by the Charles Rivers Mice Farms, Inc. This strain was relatively homozygous, long lived and quite vigorous. The individuals showed excellent uniformity in weight gain and little susceptibility to disease producing organisms. Virgin females were used to reduce possible variations attributable to resistance because of sex. The animals were used in the testing program when they reached a weight of 30-40 grams. They were maintained in self-cleaning wire cages. The mice were fed Purina Dog Chow[1] each day and a head of lettuce was placed in each cage twice a month. Water was available at all times. The cage room temperature was maintained at 86 F ±3.

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[1]Made by Ralston Purina Company, General Offices, Checkerboard Square, St. Louis, Mo.

The cockroach colony was established from laboratory colonies in the Oklahoma State University Department of Entomology Insectary. It was maintained in a 20 gallon garbage can filled with wooden shelves. The animals were fed Purina Dog Chow and water was available at all times. The temperature was held at 86 F ±3.

Laboratory Materials

Spider Holding Cages. Two procedures were used in the maintenance of the spiders under investigation. The species, such as L. reclusa, that were under detailed investigation were kept in large numbers. These species were held in pint Mason jars with double screen lids. Larger species were held in various sized paper cartons with cloth-screen tops. The spiders were all maintained at the same temperature as the test animals. Insects were placed in each cage once a week. A few drops of water were added to the cages each week for the species that required free water. Because many spiders were cannibalistic, it was necessary to provide each spider with its own cage.

Field Collection of Spiders. During the survey for new potentially dangerous species, the specimens were field collected by placing a large shell vial (9cm X 2.5cm) in front of each animal. A cotton plug was then moved in behind the spider. This caused the animal to run into the vial and the plug was inserted. Since each animal had to be collected in a separate container, the use of the vials allowed for a large number to be carried at one time. The vials were also large enough to be used in holding the spiders in the laboratory; thus, eliminating the task of transferring each spider to a larger container. When the spiders were moved to the laboratory, they were given food and water and held for one week before study. This delay was used to

reduce venom variables resulting from a lack of food or from recent depletion of the venom supply.

The survey species were collected from June to November, 1966. This time period was chosen because of the possibility that venom strength varied during the year and this was the period when the black widow venom was found to be most toxic.

Venom Recovery Equipment. The major piece of equipment used in the "milking" procedure consisted of an electrical stimulating device known as an audio generator (Heathkit model IG-72) and positive and negative electrodes. The companion equipment consisted of holding cells which were used to keep the animals immovable; small pieces of cigarette paper used to collect the venom; a dissecting microscope to observe the spider; and a Mettlar Digital analytical balance (model H 16) to weigh the venom.

Testing Procedure

Venom Recovery. The same basic technique was used throughout the study. The spider to be "milked" was anesthetized by introducing CO_2 directly into the spider's cage through a tube. The spider was then placed in a plastic cell under the microscope and the venom extracted by the technique described by Grothaus and Howell (1967). When the amount of venom obtained was too slight to be weighed, it was necessary to "milk" several animals and pool the toxin. If only one such specimen was available, the weight could only be recorded as less than .00001 gm. In a few tests it was desirable to study the effects of direct bites, in which case each spider was placed in a holding cell and pushed against the test animal, while a second person applied the electrodes to the intersegmental membrane between a femur and patella

of the first pair of legs.

Venom Injection. After the venom had been collected, it was combined with normal saline or distilled water depending on the test. The venom from a spider not previously studied was always added to 0.1 cc of distilled water and drawn into a 0.25 cc syringe. The material was then injected into a mouse using a 26 gage needle. This needle produced no observable tissue damage when used. 0.1 cc of distilled water was used because it was the maximum amount that could be injected without causing swelling and skin damage. After the test, the same spider was held for one additional week and "milked" again if possible. The recovered venom was added to .05 cc of normal saline and injected into an American cockroach.

Injection into a mouse was accomplished by permitting the animal to crawl into a blind screen tube that was two inches long. As the mouse crawled into the tube, the hind leg was grasped by the thumb and forefinger. The cage was then held in the palm of the same hand. The procedure made it possible to hold the animal without using a restraining wire or general anesthesia. The external side of the leg was shaved from the foot to the hip. A subcutaneous insertion of the needle was made midway between those points and this was accomplished by holding the syringe almost parallel to the leg. The needle was then forced upward for about 1/4 of an inch. The injection was made and the needle removed quickly. Experimentation with different injection sites and techniques indicated that this procedure resulted in little loss of venom from the entrance puncture and also approximated an actual bite. The leg was selected as the insertion site because of its relatively large mass of muscle tissue. It was also possible to observe

localized effects to the part by studying the walking behavior of the animal.

The arthropod tests were completed by anesthetizing adult cockroaches with CO_2 and injecting the venom into their abdominal cavities. Each animal was captured from the colony cage, placed in a paper carton and anesthetized. The roach was then placed on its back and injected through the fifth abdominal intersegmental membrane midway between the ventral mid-line and the lateral edge of the sternite (Figure 1). Damage to the vital systems was avoided by injecting in this area.

Venom Evaluation. When a mouse was injected, it was marked by ear notching and placed in a special holding cage and observed at intervals of 1, 2, 5, 12, 24 and 48 hours. The animal was transferred to a larger cage after 48 hours and held for one week, after which it was re-examined for possible chronic reactions. Each mouse was used for three tests and destroyed. If a positive response was noted from a mouse that had been used in other tests, the experiment was repeated with a mouse not previously injected to eliminate the possibility that the mouse had become sensitive to spider venom. Because the animals were used only three times and never subjected to the venom of the same species of spider twice, the possibility of an immune response developing was disregarded. Shulov (1962) and others have shown that it requires about six injections of spider venom before an immune response begins to develop in small mammals.

All reactions were noted and described for further reference. All lesions were measured and the size was recorded for later use as an index of venom potential. The lesions were frequently irregular in shape, making it necessary to measure their length and width rather

than the diameter. Most of the necrotic lesions were recorded as "wet" or "dry lesions". The "wet lesions" resulted from the rapid breakdown of tissue at the injection site accompanied by an accumulation of serum. The "dry lesions" developed more slowly and were similar in appearance to tissue subjected to brown spider venom. In some tests the animals were killed and examined for the gross pathological condition of the major organs.

When venom testing was complete, the spider was killed and preserved in 90% ethyl alcohol and shipped to the spider taxonomist. When available a male, female and immature specimen of each species was collected and studied, so that a completed understanding of the venom potential could be ascertained. Records were kept on the ease and rapidity with which the various spiders released their venom. This made it possible to predict the danger of receiving a serious bite if the spider was known to be dangerous.

RESULTS AND DISCUSSION

Special Studies

Venom Injection Studies. Several experiments were made to evaluate the differences in localized reactions resulting from different injection techniques. The first work was accomplished with the black widow spider. Five mice were injected with 2.0 mg of venom and regurgitated fluid. All five mice developed "wet lesions" in one hour (Figure 2). The surface tissue was destroyed but the deeper tissue appeared uneffected. The lesions were dry and healed after 24-48 hours. The mixed fluids were used because the author was unable to obtain venom free of contamination. In an attempt to compare the different fractions, the venom glands were dissected from one adult female and macerated in distilled water. The material was then injected into the mouse in the usual way. The mouse became hypersensitive to external stimuli but no large lesion developed. In the companion test, 3.4 of regurgitated material was recovered with little or no contamination by venom. This material was injected subdermally and caused no generalized reaction. However, the animal did lose the use of the leg for two days, but no tissue damage could be observed. Upon the conclusion of these tests, direct bites were attempted. Three spiders were caused to bite three mice on the hind leg. All three mice became hypersensitive to external stimuli but the only local damage noted was some bruising of the injected area (Figure 3). One additional test consisted of placing 3 mg of venom and regurgitated fluid on the surface

of the leg with the use of a capillary tube; the skin was not broken. The mixture did not break down the epithelial tissue on the outer surface.

The variation in reactions observed in these tests was confusing, but the data showed that the localized "wet lesion" occurred only when the venom was contaminated with fluid regurgitated from the digestive system. Neither venom nor digestive fluid caused localized damage when injected separately. The combination also failed to affect the skin when applied topically. From the data it must be assumed that a different mode of action occurred when the materials were injected together.

The public health impact of the black widow spider has been considerable. However, because of its habits and relatively docile nature, it has probably been overly feared. The bite was found to be dangerous, but in the mice studies none of the animals ever died. They did show severe symptoms but invariably recovered. Clinical records indicate that in strong healthy humans, the body usually recovers with little difficulty.

After it was realized that a problem existed with contamination in the black widow spider, the experiments were widened to include members of the Lycosidae. These animals seldom regurgitated and they also provided large quantities of venom during a single "milking". The first test was made with L. carolinensis. This species was thought to cause no dramatic localized response. Two mice were injected with 4.0 mg of female spider venom. One injection was a standard subdermal injection and the second was accomplished by placing the needle at a 90 degree angle to the leg and inserting it into the muscle. The

reaction was identical in both mice. The animals became hypersensitive for about two hours and their legs became swollen at the injected area. After recovery no local tissue damage was evident (Figure 4). The companion tests consisted of using a female Geolycosa sp., probably uinticolens. The venom from these animals caused "wet lesions" when it was present in large enough volume. Two mice were injected each with 4.24 mg of venom, one injection was subdermal and the second was intramuscular. Both animals developed "wet lesions" accompanied by hypersensitivity. A third species, L. antelucana which consistantly caused lesions at the proper dosage level, was used to inflict a direct bite on the leg of a third mouse. The bite resulted in a "wet lesion" very similar to those obtained by injection (Figure 5, 6).

Except for the difference noted with the black widow, the other tests revealed little difference in clinical symptoms between animals injected intramuscularly, subdermally or exposed to direct bites. The lesions caused by subdermal injections were larger than those caused by direct bite or intramuscular injection, but this was the only difference noted.

Family Loxoscelidae. The species L. reclusa has been proven to be of public health importance; for this reason it was subjected to a variety of tests.

Approximately 300 specimens were "milked" during the study. The spiders were found to be very fragile and difficult to "milk" without injury. Adult females with egg sacs were the most aggressive. Immature spiderlings were the least aggressive. The adult females usually struck the bite pad readily, but about 25% of them refused to release venom. This variability in biting behavior has probably accounted for

some of the differences in clinical symptoms resulting from bites on humans. Many of the spiders also regurgitated while injecting venom.

Two tests were run to determine whether the regurgitated material had any effect on mammalian tissue. The liquid was recovered by placing a fine capillary tube in front of the mouth and stimulating the spider. The animal was carefully observed to avoid contamination of liquid with venom. In the first test, 1.05 mg of fluid were obtained from a female and 1.01 mg were recovered from a male. The material was transferred directly to a syringe and diluted with .05 cc of distilled water. Each aliquat was injected into the hind leg of a 30 gm mouse. No localized or systemic reactions occurred. This suggested that in this species the lesion was not caused by stomach fluids.

Venom studies were conducted in the hope of determining the venom dose necessary to cause clinical symptoms. Forty females were "milked" in this series of studies and the average venom yield per spider was 0.095 mg with a range from 0.01 to 0.20 mg. Eight different levels of venom were injected into white mice. The technique used was the same as in the survey series. The amounts were as follows: .01, .1, .12, .13, .25, .26, .3 and .48 mg. The mouse that received .13 mg of venom showed no response. All of the other animals began to behave abnormally six to twelve hours after the injections. The animals began to move restlessly around the cage, frequently licking the injection site; as time passed, they became lethargic, withdrawing to a corner of the cage and remaining immobile with eyes closed. The mice that received 0.26 and 0.48 mg of venom died during the 12 and 48 hour observation periods, respectively. The remaining animals developed small lesions about two mm in diameter at the injection point (Figure 7). The tissue

became ischemic then slowly reddened in color about six hours after the injection. The skin darkened and an open one to two mm lesion appeared in 24 to 48 hours. The lesions did not continue to enlarge, but the surface dried and hardened quickly. Healing was rapid, occurring in three to five days. A subcutaneous hardening of tissue did occur so a small lump remained beneath the skin after surface healing was complete.

Because of the lack of large lesions in the injected mice, a series of direct bites on mice was conducted. The first test was accomplished by allowing a female spider to bite the hind leg of a mouse. The mouse developed a systemic reaction similar to the symptoms in the injected mice. The symptoms first appeared at six hours and the animal died in 12 hours. The second test was designed to provide a series of mice with different quantities of venom. One very large female spider was prompted to bite five mice in rapid succession (within 15 minutes). Mice one, three and four began to develop symptoms 12 hours after the bite. Mouse three died 24 hours after the injection. Mice two and five never exhibited obvious symptoms, but all of the remaining mice developed "pin head" sized lesions by the 24th hour. The lesions were similar to those created by injection of venom except that healing appeared to be more rapid.

After this series of tests, it became apparent that the mice were not responding in a manner that had been reported in the literature. The lack of large lesions indicated that either the mice had some protective mechanism or that the amount of venom necessary to cause a typical lesion was greater than the amount necessary to cause the death of the animals. It was decided to switch test animals in an attempt to clarify the problem.

One six week old New Zealand white rabbit was obtained and the hair removed from the mid-section. The amount of 0.25 mg of female brown spider venom in 0.5 cc of distilled water was injected 1 inch below the dorsal midline, midway between the front and hind legs. Within 6 hours an area 15 mm in diameter was deep red in color. After 24 hours the area began to darken and increase in size. Necrosis of the tissue also began to occur at this time. After four days the lesion was three inches long and one inch wide and reached from the dorsal midline almost to the ventral midline (Figure 8). The black necrotic tissue was present until the 15th day when the rabbit chewed it off leaving an ulcer-like area filled with pus. After draining, the lesion scarred over in a normal manner and healing was complete in 30 days.

With the completion of this test using the same venom quantity as that which caused little reaction in mice, it was shown that the CD-1 mice were highly "resistant" to brown spider venom with respect to skin reactions. Mice injected parentically with small amounts of venom died. The reason for this reaction can only be conjectured.

In addition to the venom and regurgitated fluid, the eggs of the brown spider were also tested to see if they were toxic. Fourteen day old eggs were placed in weighing bottles and macerated in distilled water. In one test three eggs were prepared and injected into a mouse, with no response. The next test was accomplished by using 6 eggs. The only response was a slight hardening of the tissue in the injected area.

Results of these studies showed that the clinical responses were due to venom rather than other constituents in the body. They also

showed that the toxic components in the venom were not present in more than trace quantities any place other than in the venom glands.

Roaches were also tested with the venom. Amounts varying from .01 to .2 mg were injected and the knockdown time ranged from 15 minutes to three hours depending on the dosage. Death usually occurred in 24 to 48 hours. However, several roaches recovered after showing rather severe symptoms. This would indicate that the effect of the venom was on some system that could be replaced or repaired.

There can be no question as to the public health problem posed by this species. The problem in less severe because of the species' preference for quiet dark places and its reluctance to bite. The animals that do strike often do not inject venom and if they release venom, most of them release only .01-.03 mg of venom. These factors singly or in combination cause a reduction in clinical symptoms. The mechanism involved in causing local or systemic reactions has not been determined; but with some idea of the levels of venom necessary to cause various reactions, it may be possible to obtain answers through bio-chemical studies now in progress.

Because of the type of reaction caused by this species, the lack of antivenom, proven effective drugs, and the habitat of this species, it should probably be considered the most dangerous species in the United States.

Family Theraphosidae. The only known representative of this primitive family in Oklahoma is Dugesiella hentzi (Girard), the Arkansas tarantula. The adults are dark brown and are about 40 mm in length. The females spend much of their life in burrows and natural cavities. The males are commonly seen migrating in Oklahoma in early

spring and again in the fall. These specimens are usually males and are apparently searching for females. They are frequently feared by layman. However, they are usually regarded as non-poisonous by persons having access to the literature.

The "milking" procedure for these animals was similar to the general technique, but it was necessary to use a high voltage for a greater period of time. These animals struck the pad readily, even biting so hard that they broke the fangs, but they seldom released venom until they had been irritated for several minutes. The venom yield varied from 0 to 15 mg and the average yield from 11 spiders was 7.39 mg. The mice studies consisted of 10 injections using varying venom quantities. Two mice received 2.10 mg of female venom and recovered. One mouse receiving 3.11 mg of male venom was dead in three hours. One receiving 10.06 mg of male spider venom died in 2 hours. Three mice received 3.4 mg of female spider venom each and two recovered while one died in two hours. When the quantity was increased to 14.5 mg, the three mice treated all died within two hours. This would indicate that the LD50 lies somewhere between 100 and 300 mg/kg. The roach study consisted of two tests, one with 0.35 mg of venom and one with 0.07 mg. The first roach was paralyzed in 15 minutes and died within 30 minutes. The second roach was not affected. A special test was conducted to see if tarantula venom became weaker if the supply was exhausted or if the animals replenished the glands with equally toxic venom. Four spiders were "milked" each day for three days and the venom pooled and injected into mice. Enough venom was released the first day to treat three animals with 10 mg of venom each; all three mice died. The second day a total of 10 mg was collected

from all three spiders. This was injected into a mouse and caused its death. The third day only 3.5 mg of venom was recovered and injected. This amount also caused the death of the mouse. Results of this experiment showed that the animals were not diluting the venom but simply releasing less material of similar potency as the glands became empty.

The data in general shows that this spider cannot be considered harmless. It is probable that humans are less sensitive to the venom than are mice but with the potential venom dosage as high as 15 mg a severe reaction could occur, especially in individuals sensitive to arthropod toxins. There is some question as to the properties of the venom and O'Dell, Nathez, Grothaus and Howell (unpublished data) suspect that the venom is not of a protein nature. Disk electrophoresis studies have, so far, failed to indicate any protein fractions and the mechanism of toxicity is unknown. When mice were exposed to the venom, they first exhibited extreme hypersensitivity to sound or touch and then became convulsive before death so it is possible that this material affected the nervous system.

Survey Data

The data for the survey portion of this research are presented first and are arranged by families according to the arrangement of Gertsch (1949). The generic and specific entries are arranged alphabetically. Each species entry is preceded by a number which corresponds to the collection number listed in the appendix. Two entries with the same number indicate that the same spider was used in two experiments. The code used in the tables is as follows:

Kd. -- time after treatment at which roaches were unable to turn over after being placed on their backs.

D. -- death.

Neg. -- no observed response to venom.

W. L. -- "wet lesion" at injection site.

D. L. -- "dry lesion" at injection site.

Sw. -- abnormal swelling at injection site.

Sys. -- systemic poisoning symptoms.

No Bite -- animal refused to strike and release venom.

imm. -- immature specimen, which was not sexed.

sp. -- specimen.

♂ -- adult male.

♀ -- adult female.

? -- material other than venom was also recovered.

Family Oecobiidae. This is a relatively small family and the known species are very small (less than 5 mm). These animals may be found on the window sills of buildings. The only species collected during this study was Oecobius texanus Bryant. Several specimens were studied, but they never released venom. They had extremely small chelicerae and were unable to pierce the epithelium on the investigator's forearm.

Family Uloboridae. Members of this family weave orb webs and remain on them, attacking prey that becomes entangled. The genus Uloborus is common in Oklahoma and can be recognized because they invariably construct their webs in a horizontal plane, while other orb weavers commonly place the web in a vertical position. The members

of the family occupy a variety of habitats varying from open buildings to woodland situations.

Venom glands have not been recognized in any of the species. The individuals apparently kill their prey by repeated piercing with the chelicerae. One species in this family was studied to discover if the salivary or digestive fluids were in some way capable of causing a toxic reaction. One female (53-S) Uloborus glomosus (Walckenaer) was "milked" and less than .01 mg of released material was recovered. The material caused neither a local or systemic reaction when injected into a mouse. This indicated that the animal had no toxic fluids.

Family Pholcidae. The spiders in this group are long-legged and hang on irregular webs in an inverted position. They are found in dark locations, cellars and "crawl spaces" being their favorite habitats.

The species, Pholcus plalangoides (Feusslin), was studied because of its frequent occurrence in the state. The specimen "milked" (23-S) was a female that yielded 0.09 mg and 0.02 mg of venom in two separate experiments. The first aliquot of venom was injected into a mouse with no results. The second aliquot was used for the roach test. No response was seen in the roach until the twelve hour observation was made. At that time the animal was on its back and death occurred within 48 hours. The roach response indicated that the venom had some toxicity; however, because of the long period of time necessary for the reaction to occur, it was doubtful if the venom was of importance from a public health standpoint. Because of the difference in reaction between the arthropod and mouse test, it was possible that the venom fraction responsible for arthropod toxicity was not harmful to mammalian tissue.

Family Theridiidae. The species in this family build an irregular web from which they usually hang awaiting their prey. When an arthropod becomes entangled, the spider quickly entangles it with silk until it can no longer move. The spider then proceeds to bite the prey and inject venom. The family is a very large one. The species are frequently found in houses or in low shrubbery near houses.

TABLE I

VENOM YIELD AND INJECTION RESULTS FROM SPECIMENS IN THE FAMILY THERIDIIDAE

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
37-S	Latrodectus mactans(Fabr.)♀	6.80?	W. L.		
100-S	Theridion goodnightorum Levi ♀	0.01	Neg.		
115-S	" " " ♀	0.00	No Bite		
5-S	Achaearanea tepidariorum(C.L.Koch)♀	9.14?	W. L.		
40-S	" " " ♀	0.70	Neg.		
40-S	" " " ♀	0.60		1 hr	48 hr
54-S	" " " ♀	0.31	Neg.		
54-S	" " " ♀	1.87		1 hr	48 hr
85-S	" " " ♀	0.74	Neg.		
85-S	" " " ♀	0.36		Neg.	Neg.
156-S	" " " ♀	0.75	Neg.		
156-S	" " " ♀	0.64		Neg.	Neg.
2-S	Steatoda triangulosa(Walck.)♀	0.02	Neg.		
2-S	" " " ♀	0.05		Neg.	Neg.
47-S	" " " ♀	0.00	No Bite		

The family Theridiidae contains the infamous black widow spider (L. mactans). The species was discussed in detail on previous pages, but one spider was "milked" and included in the survey to aid in comparative discussion. The spider used in experiment 37-S provided 6.80 mg of fluid, but much of the material was expelled from the buccal cavity. The mixture was injected following the standard procedure. The injected fluid caused the most spectacular lesion observed during the entire period of research. The skin of the injected leg began to appear abnormal within 15 minutes after the injection. Swelling was pronounced and the leg became deep purple in color. Within one hour, the epithelium began to disintegrate. The lesion continued to enlarge for another hour. The maximum size was 10 x 20 mm, and covered most of the leg (Figure 2). Serum was liberated for 12 hours and then the lesion began to dry. This same response was observed in another species when mixed fluid was injected (5-S). The mixture caused a greater local reaction than that which occurred when only venom was recovered. All of the animals in the family expelled fluid from the buccal cavity when more than the slightest stimulus was applied. One would expect the same response to occur if the animals were placed under natural stress, but this apparently does not occur. It was reported in case histories studied that black widow bites caused a systemic reaction but no localized lesion. However, a "wet lesion" has occurred experimentally so it could occur and be improperly diagnosed.

The roach tests showed that the venoms of the species studied had a rapid knockdown potential but were very slow in killing the animals. Generalizations concerning the family are not possible because of insufficient data. The data available indicate that when pure venom

is injected in normal amounts there is little reaction in either mice or roaches; the exception being the genus Latrodectus.

Family Argiopidae. Most of the American species build snares in the form of an orb and hang on or near them waiting for their prey to become entangled and helpless. They may be found wherever suitable web sites occur. The family is large and contains species of all sizes. The group is most easily identified by the orb type web.

TABLE II

VENOM YIELD AND INJECTION RESULTS FROM SPECIMENS IN THE FAMILY ARGIOPIDAE

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
15-S	Acanthepeira stellata(Walck.)♀	0.50	Neg.		
42-S	Argiope aurantia Lucas ♀	5.85	Sw.		
42-S	" " ♀	5.24		1 hr	6 hr
57-S	" " ♀	6.62	W.L.		
57-S	" " ♀	1.30		1 hr	48 hr
78-S	Argiope trifasciata(Forskal) imm.	1.40	Neg.		
38-S	Eustala sp. imm.	1.50	Neg.		
45-S	Neoscona arabesca(Walck.)♀	0.60	Neg.		
84-S	" " ♀	1.03	Neg.		
84-S	" " ♀	2.16		12 hr	24 hr
146-S	" " ♀	0.30	Neg.		
41-S	Neoscona sacra(Walck.)♀	1.00	Neg.		
41-S	" " ♀	0.27		Neg.	Neg.
52-S	" " ♂	0.63	Neg.		

TABLE II (Continued)

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
52-S	Neoscona sacra(Walck.) ♂	0.13		Neg.	Neg.
71-S	" " ♀	1.84	Neg.		
71-S	" " ♀	3.63		12 hr	48 hr
80-S	" " ♂	0.04	Neg.		
101-S	" " ♀	0.50	Neg.		
101-S	" " ♀	0.15		Neg.	Neg.
112-S	" " ♀	0.20	Neg.		
113-S	" " ♀	7.10?	W.L.		
117-S	" " ♀	3.40	Neg.		
155-S	" " ♀	0.45	Neg.		
55-S	Neoscona sp. imm.	0.89	Neg.		
55-S	" "	1.39		Neg.	Neg.

This group caused little reaction when their venom was injected into mice. The large garden spider, A. aurantia, did produce a "wet lesion" 5 mm sq. but was a reluctant biter. N. sacra caused a lesion 10 mm X 3 mm in one test, but the venom was contaminated with a considerable amount of extraneous fluid. Numerous tests were run on this species and no response was noted when only pure venom was injected. However, it is conceivable that when placed under extreme stress, these spiders could regurgitate as they were striking and in this way introduce the fluid into the tissue. If this should occur, then the result would probably be a small, rather painful lesion. The venom evaluated

seemed to be more effective on roaches. They were immobilized in an hour with death occurring in 6 to 48 hours.

Family Tetragnathidae. This group has very long chelicerae which gives them a rather foreboding appearance. One genus commonly called the long-jawed orb weavers was studied. One male Tetragnatha laboriosa Hentz and one immature Tetragnatha sp. (158-S, 4-S) were tested and found to be exceptionally docile. The animals refused to extend their fangs when subjected to electrical stress. Gross examination revealed that these animals had large fangs which were capable of inflicting a rather deep injection. It was somewhat surprising to find that they did not make more effective use of their fangs. It is possible that the fangs have become too large for effective use.

Family Linyphiidae. This family has morphological characters very similar to those in the family Theridiidae. It is very difficult for inexperienced persons to identify the group. The species are all small in size, seldom more than 5 or 6 mm long. Most of them construct irregular webs in grass, low shrubs or similar type habitats. They are very shy and docile animals, relying on their web to catch small, rather weak insects for food.

The species Linyphia marginata C. L. Koch was commonly found in Oklahoma and because of its abundance, collected throughout the warmer periods of the year. "Milking" was attempted throughout the year with a variety of specimens, but the animals became immobile and died without attempting to bite. It is doubtful if any of the species could pierce human skin if they tried.

Family Agelenidae. The members of this family are sheet-web weavers. They commonly construct a tight flat web with a funnel at one

edge. The spiders run on top of the web and retreat to the funnel when disturbed. They are frequently found in grass, forest litter and under the bark of trees.

Two genera were collected in this study and a total of five individuals were "milked". One specimen in the genus Coras was collected but was immature and could not be determined to species.

TABLE III

VENOM YIELD AND INJECTION RESULTS FROM SPECIMENS IN THE FAMILY AGELENIDAE

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
51-S	Agelenopsis emertoni Ch. & Ivie ♂	0.71	Neg.		
51-S	" " " ♂	0.25		Neg.	Neg.
114-S	Agelenopsis naevia(Walck.) ♀	4.45	W.L.		
114-S	" " " ♀	0.26		Neg.	Neg.
36-S	" " " ♂	0.54	Neg.		
124-S	Coras sp. imm.	0.16	Neg.		

One experiment in mice resulted in a positive local reaction. No systemic responses were observed with this family. A female, A. naevia, was responsible for a round 6 mm typical "wet lesion". The lesion developed rapidly, reaching its greatest size at 2 hours; at 24 hours, the lesion was dry and it was healing by the 48th hour. The epithelium disintegrated rapidly accompanied by weeping. Underlying tissue was not visibly affected. The serum was red in color, indicating either a loss of red blood cells or free hemoglobin. The mouse held the leg

up refusing to walk on it, which indicated that localized pain accompanied the disintegration of epithelium.

The "milking" data indicated that when these spiders bit they seldom used their complete supply of venom. But one animal did release a large amount of venom, so their potential to create localized lesions is possible but reduced by the apparent tendency to retain the major portion of the venom supply even when biting under stress.

Family Oxyopidae. These animals are easy to recognize because of the great number of long spines on the legs. They live in vegetation and capture their prey by stealth.

TABLE IV

VENOM YIELD AND INJECTION RESULTS FROM SPECIMENS IN THE FAMILY OXYOPIDAE

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
16-S	Oxyopes salticus Htz. ♂	0.00	No Bite		
58-S	Peuctia viridans Htz. ♀	0.42	Neg.		
58-S	" " ♀	0.50		6 hr	12 hr
83-S	" " ♂	0.10	Neg.		
163-S	" " ♀	1.71	Neg.		
163-S	" " ♀	0.30		Neg.	Neg.

Two species were studied and found to cause no response in mice. The O. salticus specimens refused to bite. This species is very small and probably relies on size to avoid enemies. P. viridans caused the death of a roach in 12 hours; when the amount of venom was reduced, no

reaction occurred. This species has been reported in the literature as a possible dangerous species. The kill time on the roach was extended, but a response did occur. The mouse data support the conclusion that this species is not a particularly dangerous one.

Family Pisauridae. The pisaurids are hunting spiders and do not build webs. One curious genus, Dolomedes, contains the fishing spiders which feed on aquatic insects and, on occasion, small fish that venture near the water margins. The other members of the family are found in tall grass and other dense vegetation.

TABLE V

VENOM YIELD AND INJECTION RESULTS FROM SPECIMENS IN THE FAMILY PISAURIDAE

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
126-S	Dapanus mirus(Walck.) imm.	0.00	No Bite		
141-S	" " imm.	0.50	Neg.		
141-S	" " imm.	1.20		24hr	48 hr
148-S	" " imm.	0.25	Neg.		
148-S	" " imm.	0.20		Neg.	Neg.
89-S	Dolomedes triton sexpunctatus Htz.♀	2.35	Neg.		
129-S	Dolomedes sp. imm.	0.00	No Bite		
145-S	" sp. imm.	0.09	Neg.		
145-S	" sp. imm.	0.05		Neg.	Neg.
128-S	Thanatidius tenuis (Htz.) imm.	0.00	No Bite		

Only one positive reaction occurred in this family. D. mirus venom caused a reaction in the roach test. The roach was on its back 12 hours after the injection and dead at 48 hours. This species is rather small and normally utilizes animals smaller than American cockroaches for food, so the venom is probably quite effective on smaller animals. Several other tests were run with this species and the spiders either refused to bite or released only a small amount of venom. All of the specimens were small and immature.

Family Lycosidae. This is a large group containing species of varied sizes. In general, these animals are moderate to large in size and hunt their prey rather than construct snares. They are commonly found hunting at night. Most of them live on the ground, being found in all types of ground habitats. Because of their hunting habits, they are referred to as wolf spiders. They have few obvious morphological characters which would enable one not familiar with spiders to identify them.

Because of their size, hunting habits and prevalence around domestic situations, this group was studied in more depth than any of the other families.

TABLE VI

VENOM YIELD AND INJECTION RESULTS FROM SPECIMENS IN THE FAMILY LYCOSIDAE

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
74-S	Arctosa littoralis (Htz.) ♀	1.00	Neg.		
140-S	" " ♀	1.14	Neg.		

TABLE VI (Continued)

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
140-S	Arctosa littoralis (Htz.) ♀	1.27		24 hr	48 hr
144-S	" " ♂	0.36	Neg.		
144-S	" " ♂	0.21		Neg.	Neg.
120-S	Geolycosa uinticolens(Ch.) ♀	3.44	Sys.		
120-S	" " ♀	3.3		1 hr	2 hr
119-S	Geolycosa sp. ♀	3.76	W.L.		
119-S	" sp. ♀	1.32		1 hr	2 hr
161-S	" sp. ♀	3.00	Sys.		
8-S	Lycosa antelucana Mont. ♂	1.06	Neg.		
24-S	" " ♀	2.14	Sw.		
24-S	" " ♀	1.17		Neg.	Neg.
27-S	" " imm.	4.51	Sw.		
33-S	" " ♀	6.40	W.L.		
33-S	" " ♀	1.79		1 hr	6 hr
43-S	" " imm.	0.75	Neg.		
43-S	" " imm.	1.00		1 hr	2 hr
44-S	" " ♂	0.73	Neg.		
56-S	" " ♂	0.57	Neg.		
81-S	" " ♀	3.63	Neg.		
81-S	" " ♀	0.50		6 hr	12 hr
103-S	" " ♀	1.71	Sw.		
109-S	" " ♀	1.10		6 hr	12 hr
149-S	" " imm.	0.70	Neg.		

TABLE VI (Continued)

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
159-S	Lycosa antelucana Mont. ♀	1.92	Sw.		
159-S	" " ♀	2.82		4 hr	6 hr
17-S	Lycosa carolinensis Walck. ♀	5.32	Sys.		
17-S	" " ♀	2.15		1 hr	24 hr
151-S	" " ♂	3.69	Sw.		
151-S	" " ♂	5.10		1 hr	2 hr
150-S	Lycosa gulosa Walck. ♀	0.31	Neg.		
150-S	" " ♀	0.20		Neg.	Neg.
125-S	" " imm.	0.00	No Bite		
108-S	Lycosa helluo Walck. ♀	0.00	No Bite		
118-S	" " imm.	6.81	W.L.		
118-S	" " imm.	3.00		1 hr	2 hr
127-S	" " ♂	0.42	Neg.		
136-S	" " imm.	0.55	Neg.		
82-S	Lycosa punctulata Walck. imm.	1.35	Neg.		
82-S	" " imm.	0.35		Neg.	Neg.
7-S	Lycosa rabida Walck. ♂	0.52	Neg.		
10-S	" " ♂	6.55	W.L.		
13-S	" " ♂	0.55	Neg.		
22-S	" " ♂	2.34		6 hr	24 hr
25-S	" " imm.	2.85	Sw.		
25-S	" " imm.	1.82		12 hr	24 hr
99-S	Lycosa sp. imm.	3.95	Neg.		

TABLE VI (Continued)

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
99-S	Lycosa sp. imm.	3.00		12 hr	24 hr
123-S	" sp. imm.	0.80	Neg.		
131-S	" sp. imm.	0.10	Neg.		
97-S	Pardosa lapidicina Em. imm.	0.00	No Bite		
96-S	Pardosa pauxilla Mont. ♂	0.00	No Bite		
93-S	" " ♀	0.00	No Bite		
1-S	Schizocosa avida (Walck.) ♂	0.70	Neg.		
9-S	" " ♂	4.39	W.L.		
9-S	" " ♂	0.04		Neg.	Neg.
72-S	" " ♀	0.91	Sys.		
122-S	" " imm.	0.13	Neg.		
121-S	Schizocosa sp. imm.	0.00	No Bite		
77-S	" sp. imm.	0.04	Neg.		
132-S	Trochosa avara Keys imm.	0.07	Neg.		
133-S	" " ♂	0.04	Neg.		
135-S	" " ♀	0.00	No Bite		

One species in the genus Artosa was studied. This species was relatively large but the venom yield was small. The venom had no effect on mice but one injection of 1.27 mg of venom caused the death of a roach in 24 hours. Because of the length of time required for mortality, a low toxic hazard is indicated. The effect of larger quantities of venom is not known; however, it is obvious that this

species is reluctant to release venom. The result is a species of little public health importance, regardless of the toxicity of the venom.

The genus Geolycosa is unique because these large, robust spiders live in burrows, grabbing prey near the tunnel entrance. The animals tested gave a consistently higher venom yield than other lycosids studied, but the reactions were similar. The venom caused "wet lesions" and often localized swelling occurred in the injected tissue accompanied by a systemic effect. The reaction appeared to be governed by the volume of venom received. The injected mice began to exhibit symptoms in about 12 to 15 minutes. They licked the injection site, indicating localized pain. This was followed by a period of hypersensitivity. After one hour, the animals became inactive, retreated to a corner of the cage and elevated their backs. Recovery was complete in 8 to 12 hours. Because of the obvious systemic response, the mouse in experiment 161-S was killed and posted 6 hours after injection. No gross pathological conditions were observed. The lack of damage to the organs was somewhat surprising; but it did indicate that the venom was not markedly hemolytic in action. The lack of deep tissue damage at the injection site substantiated this conclusion. The effect of the venom on roaches was positive, all animals were dead in less than 2 hours, providing evidence that the venom was very effective on arthropod systems.

The genus Lycosa is a very large genus and contains most of the larger spiders in the family. These animals are principally hunters and wanderers which explains their frequent occurrence in and around homes. The species L. antelucana was studied in detail. It was found

in abundance near buildings in the Stillwater area. With the exception of one test, a positive response occurred in mice when the quantity of venom injected was above 1 mg. When the dosage was between 1 and 4 mg. the clinical symptoms were similar to the response noted in the genus Geolycosa; and when the quantity was above 4 mg., a rapid localized necrosis occurred at the injection site. In experiment 33-S, a 5 mm sq. lesion was present one hour after injection. The epithelium was completely destroyed and serum was freely liberated. The lesion continued to grow and weep for 6 hours (Figure 9). Twelve hours after the injection, the lesion was 12 x 5 m. It was depressed about 2 mm (Figure 10) in the center. The leg was functioning normally after 48 hours and there appeared to be no muscle damage (Figure 11). By the sixth day, the lesion was reduced in size and healing rapidly (Figure 12). The lesion was completely healed in 13 days (Figure 13). The response in this experiment was typical of several of the species under study and seemed to be governed by the volume of venom injected, with the speed and size of lesion formation, increasing as the quantity increased. Experiment 81-S proved to be an exception, but the venom in this study was probably contaminated with regurgitated fluid. The roach studies revealed that the venom of this species was relatively slow acting in comparison with other members of the family. All of the positive tests terminated in the death of the insects between 6 and 12 hours after injection.

L. carolinensis was studied because it had been mentioned in the literature as a possible dangerous species. No lesions were formed but a systemic poisoning response did occur. The mouse from experiment 151-S was posted at 6 hours, but no gross pathological damage could be

found. One roach received 5 mg of venom and died in 2 hours; the roach receiving 2.15 mg lived for 24 hours. Two possibilities are evident concerning this species; the venom is similar to other Lycosa sp. but is not as concentrated or a difference in venom type exists. Because of the taxonomic position of the species it is my opinion that the species has venom similar to that of L. antelucana but less concentrated.

The data on the other lycosids followed the same pattern as that encountered with L. antelucana. "Wet lesions" occurred when the venom level reached 4 mg. The Pardosa sp. did not respond to the "milking" procedure but the members of this genus are small and docile. In general, the lycosids bit readily when stimulated.

The large ones seldom released venom on the first strike. These animals have very large and powerful fangs and these are undoubtedly sufficient for protection and capture of prey.

Family Gnaphosidae. This group consists entirely of hunters which live in a small tubular web under stones or in rolled leaves. They leave the retreat to hunt prey on the forest floor.

TABLE VII

VENOM YIELD AND INJECTION RESULTS FROM SPECIMENS IN THE FAMILY GNAPHOSIDAE

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
174-S	Drassyllus sp. imm.	0.20	Neg.		
147-S	Geodrassus phanus Ch. ♀	0.15	Neg.		
147-S	" " ♀	0.10		Neg.	Neg.
175-S	Herpyllus vasifer (Walck.) ♀	0.23	Neg.		

TABLE VII (Continued)

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
162-S	Zelotes hentzi Barrows ♀	0.05	Neg.		
162-S	" " ♀	0.03		Neg.	Neg.

No symptoms were noted in the injected test animals at the venom levels indicated. This group consists of relatively small species. However, the individuals tested were not reluctant to release venom or strike when an object was placed within their reach. They also exhibited aggressive behavior when fed, frequently attacking animals of relatively large size. They have strong chelicerae for their size and it may be that a strong venom is not needed to subdue larger prey. Certainly in the species studied there was no indication of an exceptionally toxic venom.

Family Clubionidae. The individuals in this family are frequently called two-clawed hunting spiders. They are found in leaf litter, under rocks and in rolled up leaves. They are small animals (less than 1 cm in length) and are seldom seen. They rely on stealth to capture prey in their habitat.

Because of their infrequent occurrence, only two specimens were examined.

No symptoms were observed in white mice after venom injection. Although these spiders are successful in subduing small insects, the venom of the species tested is apparently too weak to provide any effect on larger arthropods and mammals.

TABLE VIII

VENOM YIELD AND INJECTION RESULTS FROM SPECIMENS IN THE FAMILY CLUBIONIDAE

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
18-S	Castianeira amoena (C.L.K.) ♂	0.09	Neg.		
134-S	" " ♀	0.08	Neg.		
134-S	" " ♀	0.07		Neg.	Neg.

Family Anyphaenidae. The members of this family are very similar to the family Clubionidae in both morphology and habitat. They frequently move to vegetation to hunt prey and in this respect differ from the clubionids.

TABLE IX

VENOM YIELD AND INJECTION RESULTS FROM SPECIMENS IN THE FAMILY ANYPHAENIDAE

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
130-S	Anyphaena sp. imm.	0.00	No Bite		
157-S	Aysha gracilis (Htz.) ♀	0.00	No Bite		
39-S	Aysha nigrifrons (Ch. & Woodberry) ♀	0.05	Neg.		
39-S	" " " ♀	0.03		Neg.	Neg.

Only one specimen in this group released venom under stress. This can again be attributed to the small size of the species involved.

Family Thomisidae. These species are frequently referred to as crab spiders and are recognizable by the first pair of legs which are laterigrade. This gives the animals their crab-like appearance.

These spiders move about freely and catch their prey by direct attack without the use of a web. They can be found in all types of habitats but are found predominantly on vegetation.

Twelve experiments were conducted with members of this family. However, many of the specimens were immatures; so identification to species was not accomplished.

TABLE X

VENOM YIELD AND INJECTION RESULTS FROM SPECIMENS IN THE FAMILY THOMISIDAE

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
49-S	Misumenops celer (Htz.) ♀	0.20	Neg.		
49-S	" " ♀	0.50		Neg.	Neg.
73-S	" " ♀	0.70	Neg.		
98-S	Philodromus pratarius (Scheffer) ♀	0.00	No Bite		
48-S	" " " ♀	0.00	No Bite		
153-S	Philodromus sp. imm.	0.27	Neg.		
153-S	" sp. imm.	0.20		Neg.	Neg.
176-S	Tibellus vasifer (Walck.) ♀	0.00	No Bite		
173-S	Xysticus funestus Keys ♂	0.00	No Bite		
105-S	Xysticus sp. imm.	0.05	Neg.		
105-S	" sp. imm.	0.05		Neg.	Neg.

TABLE X (Continued)

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
75-S	Xysticus sp. imm.	0.20	Neg.		
75-S	" sp. imm.	0.15		Neg.	Neg.

No positive reactions resulted from the venoms tested in this group. Most of the species are small to medium sized spiders and have little venom. They also have small chelicerae which make it difficult for them to inject venom into anything other than small organisms. If one were to pool venom from many individuals, it might be possible to obtain clinical reactions in test animals; but from the data, it can be seen that the species studied released very little venom when placed under stress. Some of the animals even refused to strike. These were, in general, very small species, incapable of penetrating human epithelium. Thus, the venom of the species studied was either not very toxic or the quantity released by a single spider was too small to cause a reaction.

Family Salticidae. These animals are called jumping spiders and hunt their prey. They have keen eye sight and can jump several inches when stalking a house fly or similar insect. It is a large family with members in almost every type of habitat.

TABLE XI

VENOM YIELD AND INJECTION RESULTS FROM SPECIMENS IN THE FAMILY SALTICIDAE

Sp. No.	Species	Mg Venom Obtained And Injected	Reaction To Venom		
			Mouse	Roach	
				Kd.	Death
152-S	Eris marginatus (Walck.) ♂	0.31	Neg.		
152-S	" " ♂	0.25		½ hr	1 hr
102-S	Habronattus cornatus (Htz.) ♀	0.26	Neg.		
76-S	Metacyrba taeniola (Htz.) ♀	0.08	Neg.		
14-S	Phidippus ardens Peckham ♂	1.50	Sw.		
32-S	" " ♂	0.11	Neg.		
32-S	" " ♂	0.10		1 hr	12 hr
104-S	" " ♂	0.36	Neg.		
104-S	" " ♂	0.65		2 hr	6 hr
154-S	" " ♂	0.14	Neg.		
79-S	Phidippus audax (Htz.) ♀	0.90	Neg.		
79-S	" " ♀	0.25		1 hr	6 hr
26-S	Phidippus clarus Keys ♂	0.84	W.L.		
26-S	" " ♂	0.40		1 hr	6 hr
46-S	" " ♀	1.30	Sw.		
46-S	" " ♀	0.23		1 hr	6 hr
50-S	Phidippus pius Scheffer ♀	1.35	Neg.		
50-S	" " ♀	1.26		1 hr	4 hr

The study of this group resulted in two positive reactions on mice. The venom from experiment 14-S caused localized swelling in the injec-

tion area. No necrosis was observed, but the swelling persisted for 48 hours; this is considerably longer than it would have persisted if only saline or distilled water had been injected. One species (26-S) caused a small localized lesion on a mouse. The injection site became swollen and a 4 mm sq. lesion appeared in 12 hours. The lesion was slightly depressed in the center. The margin of the lesion was bluish-red in color, indicating that localized hemorrhaging was taking place in the tissue surrounding the injection site. The reaction stabilized in 48 hours and the tissue began to heal. All of the roach tests were positive. The tests were especially significant because most of the roaches were dead in less than 6 hours. This would indicate that the venom was very toxic to arthropods. Because of the reactions obtained from the species studied, it is probable that the larger jumping spiders are capable of causing clinical reactions in both normal and hypersensitive humans.

Human Reactions

Although no specific clinical studies were made on humans, several specimens were received which had caused clinical reactions in humans. One adult female of Phidippus audax, a common jumping spider was recovered after it had bitten an elderly man behind the ear. Pronounced swelling persisted in the mastoid area for several days, causing localized pain and severe discomfort. A second report was received concerning a 9 year old girl that had been bitten on the left index toe as she placed her foot into a shoe. The spider was identified as Trachelas tranquillas (Htz.), family Clubionidae. The girl suffered localized swelling and pain and was admitted to the hospital overnight for treatment of clinical symptoms. However, the attending physician reported,

"No unusual symptoms following the bite, particularly symptoms of poisoning." This appears to represent a problem concerning the term poison. A localized reaction apparently did occur, but without a general systemic response. A third species, Trochosa acompa Ch. & Iv. was submitted by a women who claimed it bit her on the leg while she was in bed. She claimed the leg "swelled' to two or three times normal size" and became very painful.

Since all of the cases reported could have resulted from hypersensitive individuals, no general conclusions could be made. More clinical data will be needed before a valid evaluation can be conducted.

Generalizations

When attempting to evaluate the public health hazard of spiders, several aspects must be considered. In this study it became obvious that many spiders were so small that they were unable to pierce human skin with their fangs. It is doubtful if any of the species under 5 mm in length could pierce normal human skin. Thus, some of the largest families are precluded from having any possible effect on human health regardless of individual venom toxicities. Other families are of no importance because their members contain no venom glands. By eliminating the spiders in these categories, at least 60% of the known species can be disregarded. The remaining species must be subjected to further evaluation. Because they have both venom and the ability to inject humans, their importance must be evaluated on the type, strength and quantity of venom available. Their importance is further influenced by their habitats in relation to human habitats and by their reluctance to bite and release venom. Ideally, all of these factors should be used in reaching conclusions concerning specific species. The type of venom

released by the various species constitutes one of the least understood factors. Analyses of biologically active compounds are extremely complicated and costly. Fortunately, however, dangerous spiders can be located and identified without knowing this information.

In generalizing, the factors influencing the effect of spider venom in humans fall under two headings, ecology and evolution. The development of venom characteristics must be closely associated with the evolution of the species concerned. There are many criteria for placing spiders in their respective phylogenic positions, but one of the easiest to understand is the degree of specialization found in the webs. The hunting spiders construct few or no webs and are less highly evolved than the orb weavers that construct geometric webs. The spiders in between construct various types of webs which vary in complexity. If we project back to the very primitive spiders such as the tarantula, we find the Oklahoma species does not have a proteinacious venom and, although the material should not be considered non-toxic, when it is compared on a weight to weight basis, it is probably not as toxic as most other spider venoms. This could be the result of several factors, but it is probable that this species, having evolved fangs almost 1/2 inch in length, was released from selection pressure on its venom. The food normally captured can easily be killed without the use of venom. Hence, the venom is not highly toxic and possibly more important, the animals are extremely reluctant to release venom even if they are forced to strike. This same principle is involved in the larger hunting spiders because the large wolf spiders bite readily but release venom reluctantly. The medium-sized hunting spiders probably have a more toxic venom because they have not evolved to the point of building an effec-

tive web to capture prey. The jumping spiders present a better example because they frequently attack relatively large prey. If their venom was not toxic enough to quickly kill the prey, it would escape. When we study the forms that construct various types of snares to aid in obtaining food, the spiders with the poorest webs should have the most toxic venom, while the most advanced should have the least toxic venom. Because of a lack of quantitative data, this is difficult to confirm. There is one group that does reflect this trend. The species in the family Theriididae should have more toxic venom than those in the orb weaving families. The black widow genus is very toxic. This group builds less efficient webs so it may be compensating by selecting for a more efficient venom. A problem does exist, the venom of the genus is also more toxic than that found in the hunting spiders which build no webs. The only answer that would be compatible with this hypothesis, is that the group underwent divergent evolution which is not presently discernible.

Until the present, the discussion has been concerned with only the venoms, but if we are interested in the public health importance we must also consider the ecology of the species. The spiders that are restricted to habitats seldom frequented by man are of little importance regardless of their venom potential. But, spiders such as the L. reclusa that adapt readily to a household environment are of great importance. Unfortunately, the larger hunting spiders, which do have an effective venom, are wanderers and often stray into the human environment and both the black widow and brown spider are frequently involved in man's habitat.

It must be realized that any broad discussion concerning a group

such as the spiders must neglect specific exceptions. In the present discussion, the exceptions are many and the gaps in the data are large. I have presented what appears to be the most logical generalizations based on experience and the available data. This in no way precludes the possibility that more data will change the entire concept of venom potential as it relates to the evolution of the species.

SUMMARY AND CONCLUSIONS

A total of 500 spiders were "milked" in 217 separate experiments. These specimens represented 18 families, 44 genera, 54 species, and 25 specimens which were identified to the generic level. The following species were found to cause localized lesions when their venom was injected subdermally into the hind legs of white mice (CD-1 strain): Latrodectus mactans, Achaearanea tepidariorum, Argiope aurantia, Neoscona sacra, Agelenopsis naevia, Geolycosa sp., Lycosa antelucana, Lycosa helluo, Lycosa rabida, Phidippus clarus and Loxosceles reclusa. The following species were found to cause a systemic effect when their venom was injected into mice: Latrodectus mactans, Geolycosa uinticolens, Geolycosa sp., Lycosa carolinensis, Schizocosa avida, Loxosceles reclusa and Dugesiella hentzi. The following species caused localized swelling at the injection site when their venom was introduced into the mice: Argiope aurantia, Geolycosa sp., Lycosa antelucana, Lycosa carolinensis, Lycosa rabida, Phidippus ardens and Phidippus clarus.

The venoms of most of the species were also tested on American cockroaches. The members of the family Salticidae had the most rapid acting venom, with death occurring less than 6 hours after injection. The lycosids also caused a high mortality in test roaches, but the venoms were slower acting than those of the salticids. Many members of other families also caused responses in roaches but no definite family level trends could be identified.

Specific studies were conducted on the brown spider (L. reclusa).

These animals were very fragile and difficult to "milk". The adult females were the most aggressive, while the immature specimens were the least aggressive. Many of the individuals refused to release venom after striking the pad while others refused to strike. The body fluids were tested in addition to venom and were found to have no visible effect on mouse tissue. The venom caused marked systemic reactions in mice, often resulting in death, but no large necrotic lesions ever developed. The venom was also tested on a rabbit and caused a 3 inch lesion which lasted 30 days. Venom yield results were collected and in a "milking" of 40 animals, the average venom yield was 0.095 mg per spider with a range of 0.01 to 0.20 mg. The variation in venom yield and type of bite aided in explaining some of the variability in clinical reports.

Several experiments were conducted with the Arkansas tarantula. The venom yield from these animals ranged from 0 to 15 mg and the average yield from 11 spiders was 7.39 mg. Various volumes were injected into white mice and the LD 50 was estimated to be between 100 and 300 mg/kg.

It was also determined that members of this species maintained the concentration of venom at a relatively high level and injected less material as the venom glands became depleted.

The black widow was found to cause a "wet lesion" when venom was contaminated with fluid expelled from the buccal cavity. This reaction did not occur when only venom or stomach fluid was injected. It was concluded that a different reaction occurred when the two liquids were mixed.

Venom injection studies were conducted to ascertain if the depth

or type of injection affected the clinical response. Species known to cause "wet lesions" were found to cause "wet lesions" regardless of the site of the injection. The venom of species known to cause only swelling and systemic reactions caused these same reactions regardless of the depth of injection. It was noted that injected venom caused somewhat larger lesions than did the same venom when it was injected by the spider.

Three species of spiders were collected after they had caused clinical symptoms in humans; they were identified as Phidippus audax, Trachelas tranquillas and Trochosa acompa Ch. and Iv. No assumptions were made concerning these species because of the possibility that the individuals were hypersensitive.

After studying the data, it was postulated that a trend in venom toxicity could be seen at the family level. The more primitive hunting spiders that did not rely on webs to obtain food, were in general more toxic than the advanced forms that prepared very efficient webs.

Any summary of the impact of the species studied on public health must be regarded with caution when data on mice and arthropods are used to predict human responses, but the data are helpful in locating species which may be dangerous. The members of the genera Loxosceles and Latrodectus have been studied in depth and are definitely dangerous. It is very probable that the species which caused lesions on mice also are capable of causing a similar reaction in humans. The species that caused only systemic effects should be regarded with caution while those causing only localized swelling should be avoided by hypersensitive individuals. The species which caused all three types of reactions are probably capable of causing clinical symptoms in all individuals.

TABLE XII

COLLECTION DATA FOR SPIDERS USED IN VENOM STUDIES

Sp. No.	Date	Locality in Oklahoma	Collector
1.	9 Jun 66	Stillwater, Payne Co.	R. Grothaus
2.	10 Jun 66	" "	" "
4.	10 Jun 66	" "	D. Arnold
5.	27 Jun 66	" "	C. Bailey
7.	28 Jun 66	Madill, Marshall Co.	D. Arnold
8.	28 Jun 66	" "	" "
9.	28 Jun 66	Maysville, Garvin Co.	" "
10.	28 Jun 66	Madill, Marshall Co.	" "
13.	13 Jul 66	Stillwater, Payne Co.	J. Martin
14.	14 Jul 66	" "	C. Bailey
15.	10 Jul 66	" "	R. Grothaus
16.	15 Jul 66	" "	" "
17.	18 Jul 66	" "	" "
19.	18 Jul 66	" "	" "
23.	20 Jul 66	Chauteau, Mayes Co.	D. Arnold
24.	23 Jul 66	Perry, Noble Co.	" "
25.	14 Jul 66	Stillwater, Payne Co.	R. Grothaus
26.	20 Jul 66	Washington Co.	D. Arnold
27.	20 Jul 66	Stillwater, Payne Co.	S. Kunz
32.	27 Jul 66	" "	D. Arnold
33.	29 Jul 66	" "	R. Grothaus
36.	4 Aug 66	" "	" "
37.	8 Jun 66	" "	" "

TABLE XII (Continued)

Sp. No.	Date	Locality in Oklahoma	Collector
38.	4 Aug 66	Stillwater, Payne Co.	R. Grothaus
39.	5 Aug 66	" "	" "
40.	2 Aug 66	" "	" "
41.	9 Aug 66	" "	" "
42.	11 Aug 66	" "	" "
43.	11 Aug 66	" "	" "
44.	11 Aug 66	" "	" "
45.	1 Aug 66	" "	" "
46.	13 Aug 66	" "	" "
47.	16 Aug 66	" "	" "
48.	16 Aug 66	" "	" "
49.	16 Aug 66	" "	" "
50.	16 Aug 66	" "	" "
51.	16 Aug 66	" "	" "
52.	16 Aug 66	" "	" "
54.	16 Aug 66	" "	" "
55.	15 Aug 66	" "	" "
56.	15 Aug 66	" "	" "
57.	18 Aug 66	" "	D. Arnold
58.	17 Aug 66	Cherokee Co.	" "
71.	20 Aug 66	Stillwater, Payne Co.	G. O'Dell
72.	19 Aug 66	" "	R. Grothaus
73.	25 Aug 66	" "	" "
74.	28 Aug 66	" "	" "

TABLE XII (Continued)

Sp. No.	Date	Locality in Oklahoma	Collector
75.	29 Aug 66	Stillwater, Payne Co.	R. Grothaus
76.	29 Aug 66	" "	" "
77.	23 Aug 66	" "	G. O'Dell
78.	29 Aug 66	" "	R. Grothaus
79.	Laboratory reared - killed 30 Aug 66		
80.	27 Aug 66	Stillwater, Payne Co.	R. Grothaus
81.	26 Aug 66	" "	" "
82.	7 Aug 66	" "	" "
83.	17 Aug 66	Cherokee Co.	D. Arnold
84.	31 Aug 66	Mayes Co.	" "
85.	29 Aug 66	" "	" "
89.	6 Aug 66	Stillwater, Payne Co.	R. Grothaus
93.	9 Aug 66	" "	" "
96.	6 Sep 66	" "	" "
97.	6 Sep 66	" "	" "
98.	6 Sep 66	" "	" "
99.	6 Sep 66	" "	" "
100.	6 Sep 66	" "	" "
101.	6 Sep 66	" "	" "
102.	7 Sep 66	" "	" "
103.	7 Sep 66	" "	" "
104.	19 Sep 66	" "	C. Bailey
105.	23 Sep 66	" "	" "
108.	7 Sep 66	" "	" "

TABLE XII (Continued)

Sp. No.	Date	Locality in Oklahoma	Collector
109.	7 Sep 66	Stillwater, Payne Co.	C. Bailey
112.	7 Sep 66	" "	R. Grothaus
113.	29 Sep 66	" "	" "
114.	29 Sep 66	" "	" "
115.	29 Sep 66	" "	" "
117.	30 Sep 66	" "	" "
118.	4 Oct 66	" "	" "
119.	4 Oct 66	" "	" "
120.	4 Oct 66	" "	" "
121.	4 Oct 66	" "	" "
122.	4 Oct 66	" "	" "
123.	4 Oct 66	" "	" "
124.	4 Oct 66	" "	" "
125.	4 Oct 66	" "	" "
126.	4 Oct 66	" "	" "
127.	4 Oct 66	" "	" "
128.	4 Oct 66	" "	" "
129.	4 Oct 66	" "	" "
130.	4 Oct 66	" "	" "
131.	4 Oct 66	" "	" "
132.	4 Oct 66	" "	" "
133.	4 Oct 66	" "	" "
134.	5 Oct 66	Jackson Co.	D. Arnold
135.	4 Oct 66	Stillwater, Payne Co.	R. Grothaus

TABLE XII (Continued)

Sp. No.	Date	Locality in Oklahoma	Collector
136.	4 Oct 66	Stillwater, Payne Co.	R. Grothaus
140.	9 Oct 66	" "	C. Bush
141.	13 Oct 66	" "	R. Grothaus
144.	12 Oct 66	Cimarron Co.	D. Arnold
145.	13 Oct 66	Stillwater, Payne Co.	R. Grothaus
146.	11 Oct 66	Texas Co.	D. Arnold
147.	17 Oct 66	Stillwater, Payne Co.	R. Grothaus
148.	15 Oct 66	" "	" "
149.	15 Oct 66	Texas Co.	D. Arnold
150.	15 Oct 66	Stillwater, Payne Co.	R. Grothaus
151.	18 Oct 66	" "	" "
152.	26 Oct 66	" "	" "
153.	25 Oct 66	" "	" "
154.	15 Oct 66	Cimarron Co.	D. Arnold
155.	27 Oct 66	Stillwater, Payne Co.	R. Grothaus
156.	27 Oct 66	" "	" "
157.	1 Nov 66	" "	" "
158.	28 Oct 66	" "	" "
159.	31 Oct 66	" "	" "
161.	10 Oct 66	" "	S. Kunz
162.	3 Nov 66	" "	R. Grothaus
163.	2 Nov 66	Carter Co.	D. Arnold
169.	9 Nov 66	Le Flore Co.	" "
171.	11 Nov 66	Stillwater, Payne Co.	R. Grothaus

TABLE XII (Continued)

Sp. No.	Date	Locality in Oklahoma	Collector
172.	10 Nov 66	Stillwater, Payne Co.	R. Grothaus
173.	15 Nov 66	" "	S. Kunz
174.	15 Jun 66	" "	R. Grothaus
175.	15 Jun 66	" "	" "
176.	20 Jul 66	" "	" "

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APPENDIX

Figure 1. The Injection of an American Cockroach with Spider Venom

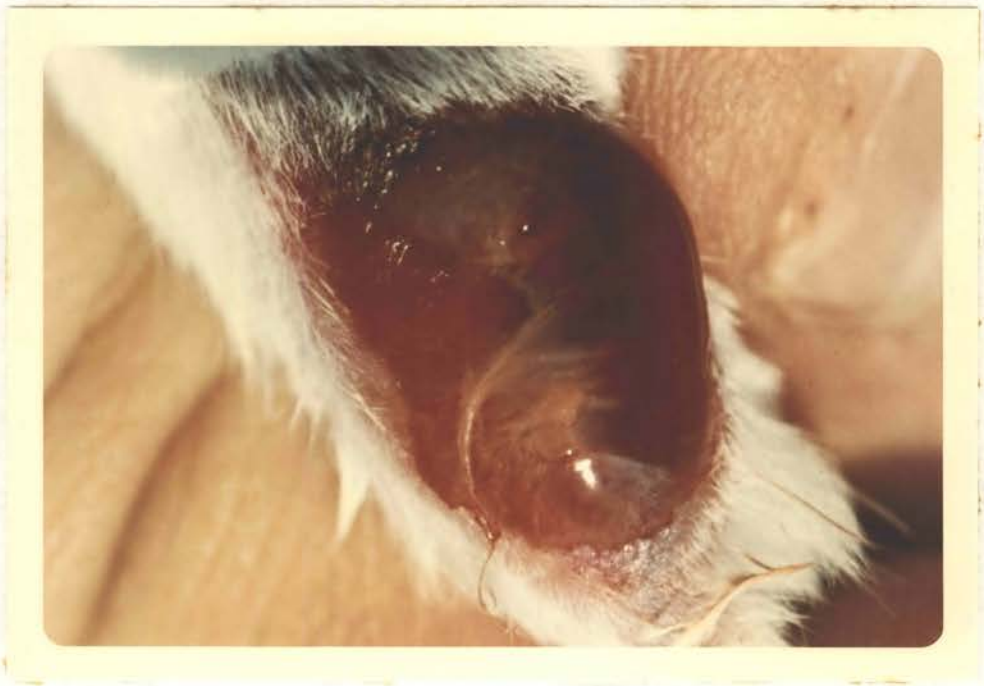

Figure 2. The Hind Leg of a White Mouse Showing the Effect of Latrodectus mactans Venom and Stomach Fluids Combined, One Hour After Injection

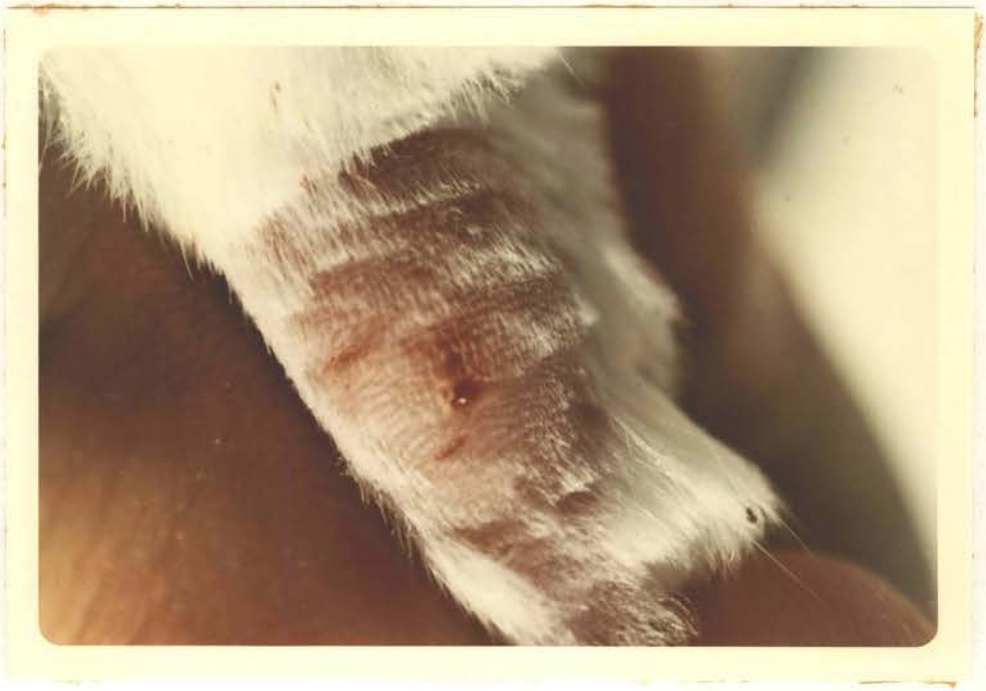

Figure 3. The Hind Leg of a White Mouse Showing the Effect of a Direct Bite from a Latrodectus mactans Spider

Figure 4. The Hind Leg of a White Mouse Showing the Lack of Damage from Lycosa carolinensis Venom

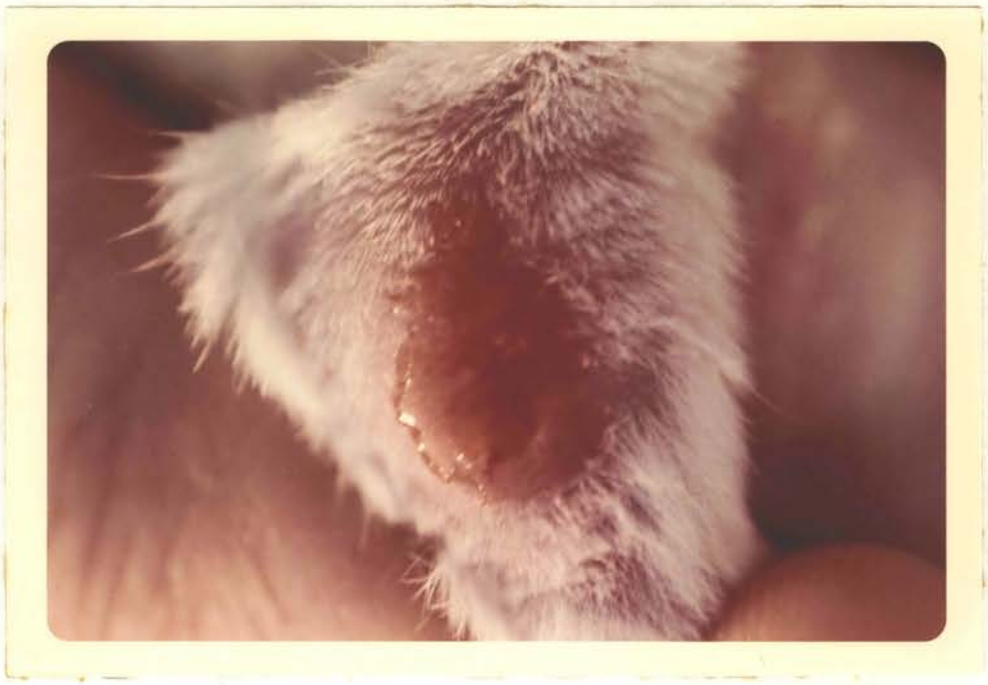

Figure 5. The Hind Leg of a White Mouse One Hour After a Direct Bite from Lycosa antelucana

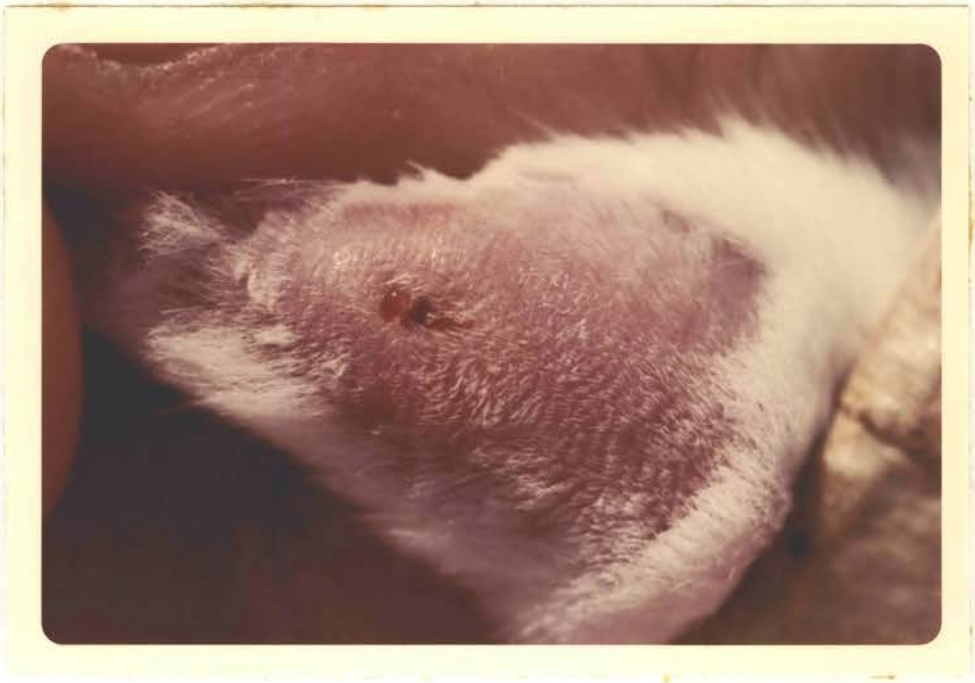

Figure 6. The Hind Leg of a White Mouse Twenty Hours After a Direct Bite from Lycosa antelucana

Figure 7. The Hind Leg of a White Mouse Showing the Lack of Response to Loxosceles reclusa Venom

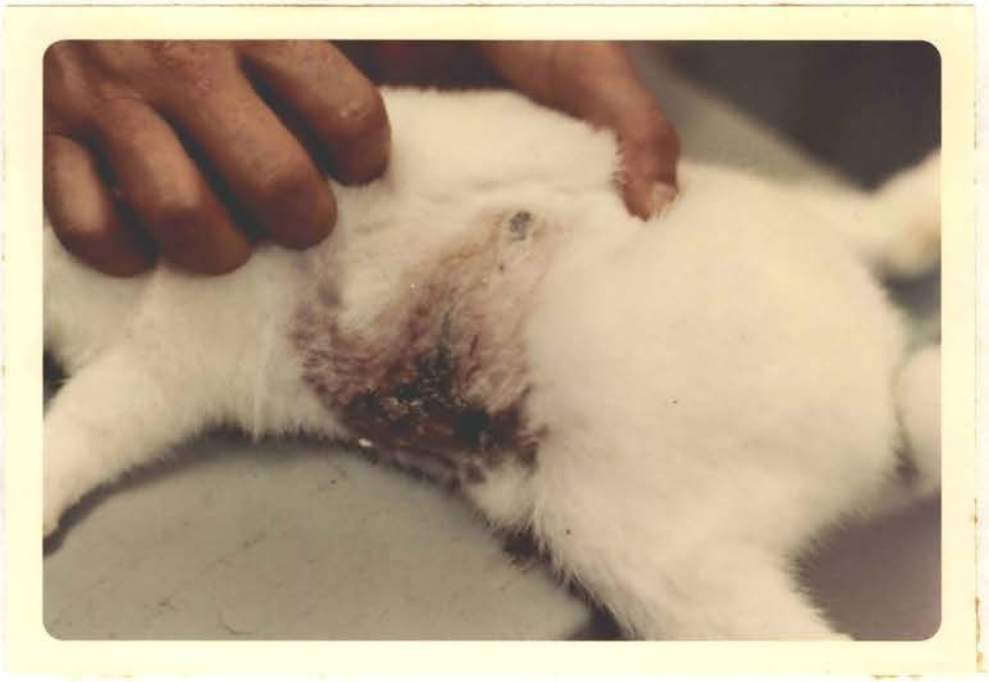

Figure 8. The Mid-Section of a White Rabbit Showing the Effect of Loxosceles reclusa Venom Four Days After Injection

Figure 9. The Hind Leg of a White Mouse Showing the Effect of Lycosa antelucana Venom Six Hours After Injection

Figure 10. The Hind Leg of a White Mouse Showing the Effect of Lycosa antelucana Venom Twelve Hours After Injection

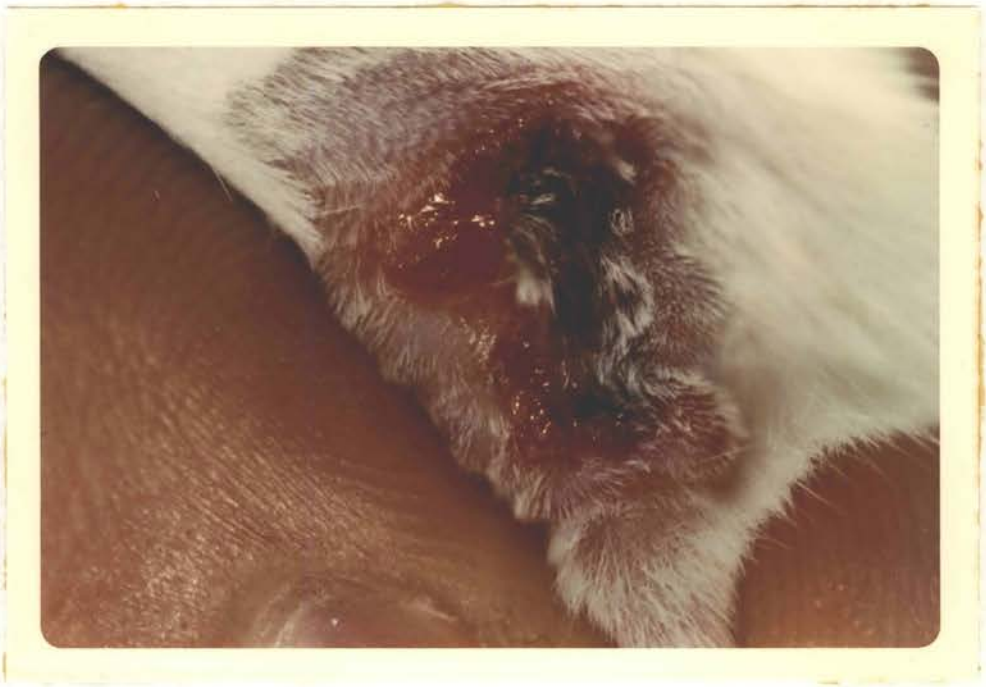

Figure 11. The Hind Leg of a White Mouse Showing the Effect of Lycosa antelucana Venom Forty-eight Hours After Injection

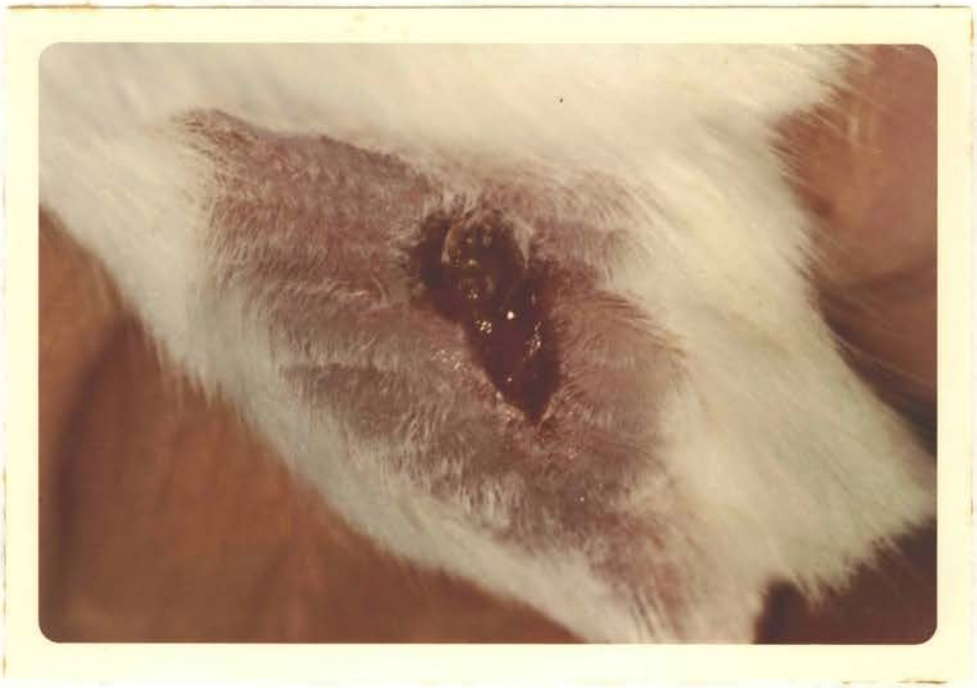

Figure 12. The Hind Leg of a White Mouse Showing the Effect of Lycosa antelucana Venom Six Days After Injection

Figure 13. The Hind Leg of a White Mouse Showing the Effect of Lycosa antelucana Venom Thirteen Days After Injection

VITA

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