THE BIOLOGY AND VENOM POTENTIAL OF THE ARKANSAS TARANTULA *DUGESIELLA HENTZI* (GIRARD)

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THE BIOLOGY AND VENOM POTENTIAL OF THE ARKANSAS TARANTULA DUGESIELLA HENTZI (GIRARD)

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PREFACE

A research problem concerning spiders and their venom was suggested by Dr. D. E. Howell, Professor of Entomology. Dr. Howell along with Dr. G. V. Odell, Professor of Biochemistry, had been involved with spider venom research for several years prior to my arrival at Oklahoma State University. Since I will be entering the Air Force as a medical entomologist, a study concerning spiders and their venom which is so important to public health seemed like an interesting study to undertake.

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INTRODUCTION

Probably the 2 spiders best known world wide are the black widows (*Latrodectus* spp.) and the tarantula (Theraphosidae). The black widows have become infamous due to serious injury or death resulting from their bites. However, the general public knows very little about the tarantula's bite. Yet, the tarantula has gained notoriety with the public due to its large size and its role as a deadly villain in movies. In recent years the American public has become increasingly interested in the public health significance of other spider bites because of the publicity given the brown spider.

I chose to study the *Dugesiella hentzi* tarantula in Oklahoma. Following the classification of Branson (1958) and Kaston (1953) all tarantulas collected in Oklahoma will be called *D. hentzi* rather than dividing this group into several spp. as some authors have done. The reasons *D. hentzi* was selected are its size, the quantity of venom that could be obtained, the possibility of obtaining pure venom, and the fact that it has not been as extensively studied as the brown spider and black widow spider. Recent workers have called tarantula venom relatively harmless to humans. Some authors even have suggested that tarantulas do not use their venom to aid in capturing prey.

To answer some of the questions surrounding the tarantula, my first objective was to collect *D. hentzi* and learn as much as possible about its development and ecology. The second objective was to determine the level of *D. hentzi* venom toxicity to mammals and insects.

All previous work on spider toxicity had not used a measured volume of pure venom. The third objective was to evaluate the pathologic effects of venom on mammalian tissues. The fourth objective was to separate venom protein components and to locate the toxic components.

REVIEW OF LITERATURE

Few authors have specifically studied the Arkansas tarantula, Dugesiella hentzi (Girard), venom and published information concerning it. However, other animal toxins have been thoroughly studied and some will be reviewed here to aid in understanding D. hentzi venom.

Medical Importance and History of Spider Bites

Scott (1963) estimated that about 25,000 cases of envenomizations per year in the United States result in severe injury. He further estimated that 26 of these cases result in death. He attributed 14 deaths to venomous snakes, 10 deaths to bees, 8 deaths to spiders and the rest to other venomous arthropods. In an analysis on 215 reported deaths from venomous animals in the United States which occurred from 1950 through 1954, Parish (1959) found that Hymenoptera killed 86 people, poisonous snakes killed 71 persons and poisonous spiders killed 39 persons. Parish noted that rattlesnakes (Crotalus spp.) and honey bees were the 2 most deadly venomous animals in the United States.

The first recorded spider bite in the Colonies occurred on September 3, 1726, in what is now Needham, Massachusetts, according to Thorp and Woodson (1945). They further determined the first report of any specific toxic spider was in 1812 in "Indian Doctors Dispensatory" by Peter Smith, which referred to the black widow spider.

Waldron (1968) indicated that 15 spp. of spiders, in addition to the black widow, had been implicated in 73 bites on man in Southern

California for the 11 year period 1955 to 1966. Horen (1966) reviewed 615 reported cases of poisonous spider bites with 38 deaths in 18 states of the United States up to the early 1940's.

In studying spider bites up to 1945, Thorp and Woodson (1945) found black widow spiders reported in 48 states with 32 cases of black widow bites in 17 of those states. They reported 32 deaths in California from black widow bites out of 55 for all states. Bogen (1926) claimed that 80% of 150 persons of reported poisonous spider bites in the United States since 1826 were males. He stated that the majority were bitten on the penis or adjacent parts while sitting in an outdoor toilet. O'Rourke (1956) disclosed that no fatalities due to Latrodectus mactans (F.) had been reported in Canada.

Arachnidism in the United States

At the present time probably the three most notorious spiders in the United States to the general public are the black widow (L. mactans), brown or fiddle-back spider (Loxosceles reclusa, Gertsh & Muliak), and tarantula (genus species varies). The fact and fantasy of these 3 spiders will be discussed in detail later. However, many other spider spp. may be responsible for arachnidism.

Peterson (1970) reported several spiders of the family Lycosidae capable of producing lesions on test animals. Grothaus and Teller (1968) reported a human case of spider bite by a male Lycosa miami (Wallace). The bite site of the L. miami was described as ischemic with no necrosis. Grothaus (1967) identified Phidippus audax Hentz, Trachelas tranquillas Hentz, and Trochosa acompa Chamberlin after they caused clinical symptoms in humans.

An unusual injury was observed by Tinkham (1946) while serving with the army in Florida. A soldier repairing a motorcycle saw a "green bug" that he poked with a screwdriver. He was struck in the eye from a distance of about 25 cm by a fine spray of liquid "like hot lead." The "bug" was identified as 1 of the lynx spiders (Peucetia viridans (Hentz).

After using 54 spiders spp. of 18 different families, Grothaus (1967) found that the venom of the following spp. caused localized lesions in mice: L. mactans, Achaearanea tepidariorum Koch, Argiope aurantia Lucas, Neoseona saira Walckenaer, Agelenopsis naevia Walckenaer, Geolycosa sp., Lycosa antelucana Montgomery, Lycosa helluo Walckenaer, Lycosa rabida Walckenaer, Phidippus clarus Keys and L. reclusa. He further found the following spp. to cause a systemic effect when their venom was injected into mice: L. mactans, Geolycosa uinticolens Chamberlin, Geolycosa sp., Lycosa carolinensis Walckenaer, Schizocosa avida Walckenaer, L. reclusa and Dugesiella hentzi. He also listed 6 spp. which caused localized swelling at the injection site when their venom was introduced into mice.

Symptoms of a Chiracanthium inclusum Hentz spider bite were described by Furman and Reeves (1957). Waldon (1968) reported a Thiodina sylvana Hentz spider bite case.

Latrodectism

Probably the most widely known poisonous spider in the world is the black widow (L. mactans). Baerg (1923), Blair (1934), Herms, Bailey, and McIvor (1935), and Baerg (1936) described the life cycle of the black widow. Baerg (1936) listed the symptoms of poisoning as

severe pain not only near the bite but in almost all parts of the body; some difficulty in breathing; nausea; and a low fever. In 3 days these symptoms subsided if the bite was not fatal, and there were no after effects.

Kirby-Smith (1942) and Maretic and Stanic (1954) discussed recorded case histories of black widow bites and their treatment. A cow was reported to be the victim of a female Latrodectus sp. bite by Cariaso (1962). Jacobs (1969) attributed peripheral neuritis following a black widow spider bite to the spider's poison or manifestations of a disease precipitated by it.

Levi (1958), Baerg (1959), and Abalos and Baez (1967) discussed the classification of the genus Latrodectus. Abalos and Baez (1967) pointed out the many problems of classifying the genus due to the few morphological elements shown for sp. differentiation. Four spp. of the genus Latrodectus were known to occur in North America. McCrone (1964) listed these 4 spp. as L. mactans mactans, L. geometricus Koch, L. variolus Walckenaer, and L. bishopi Kaston. McCrone (1964) also determined that all 4 North American spp. had very potent venoms with species differences in lethality. Sampayo (1943) and Tinkham (1956) evaluated the toxicity of L. mactans, L. geometricus, and L. bishopi on various animals.

Keegan, Hedeen, and Whitlemore (1960) noted a seasonal variation in black widow spider venom when injected into white mice. They found that the highest toxicity occurred in November and lowest toxicity occurred in April and May. Wiener (1956) showed that the toxicity of the red back spider (L. hasseltii Thorell) venom was 100 times greater at 0 C and 37 C than at 18 to 24 C. D'Amour, Becker and Van Riper

(1936), Shulov (1952), Keegan (1955), and Wiener (1956) worked with immunity development in test animals and the development of an antivenom.

Several studies had been made to determine the effects of black widow venom on insects. Beltini (1956) developed a low degree of immunity in house flies to injected venom of L. mactans tredecimguttatus Rossi. Wiener and Drummond (1956) found that venom of L. hasseltii injected into Drosophila caused paralysis and utilized this effect for assaying antivenom. They stated that the results agreed reasonably well with that obtained when the same serum was assayed in mice. D'Ajello, Mauro, and Beltini (1969) observed that L. mactans tredecimguttatus was capable of blocking transmission in the sixth abdominal ganglion. However, they further observed that axonal conduction was not affected by the venom. Grothaus (1967) used cockroaches in addition to mice to determine the toxic level of various spider venoms.

Necrotic Arachnidism (Loxosceles spp.)

Several spp. of the genus Loxosceles have been found to cause necrotic arachnidism. The most prevalent poisonous sp. of this genus in the United States was the L. reclusa. Hite (1964) described the natural habitat of L. reclusa and Hite, Gladney, Lancaster and Whitcomb (1966) described the biology of this sp.

Apparently the first record of a proven L. reclusa bite was reported by Schmaus (1929) in Halstead, Kansas. Schmaus described the bite on a 24 year old woman who had to be hospitalized. However, the role of L. reclusa as the causative agent of necrotic spider bite did not come to the public's attention until Atkins, Wingo, and Sodeman in

1957 pointed out the possibility. After that, many people, including James, Sellers, Austin, and Terrill (1961), Nance (1961), and Pitts (1962), recognized that the L. reclusa spider bite produced a necrotic lesion in humans. Morgan (1969), Denny, Dellaha, and Morgan (1964) and Shulov, Ickowicz, and Pener-Solomon (1962) demonstrated that L. reclusa venom could be fatal to test animals including dogs, mice, guinea pigs and rabbits, with rats the most resistant. Norment (1956) studied the effect of L. reclusa venom on crickets. Norment and Vinson (1969) concluded that L. reclusa venom or products of the venom had a lytic action on fat and muscle tissue of Heliothis virescens (F.) larvae and affected the hemolymph protein.

Macchiavello in 1937 observed that L. laeta Nicolet bite caused a "gangrenous spot" or cutaneous arachnidism on humans bitten by the spider in Chile. Macchiavello in 1947-48 further characterized the L. laeta bite. Vukusic (1962) related that an 8 year old girl from Colchaque, Chile, died 36 hr after being bitten on the arm by a L. laeta. Micks and Smith (1963) demonstrated that L. rufescens Dufour bites produced a necrotic lesion on rabbits. They also noted that there was little spp. variation in the biting apparatus of L. rufescens, L. laeta, and L. reclusa. Smith and Micks (1968) observed that rabbits allowed to be bitten by L. rufescens, L. reclusa and L. laeta died in 22-24 hr. They further observed that all rabbits when bitten by the 3 spp. developed large skin lesions with extensive peripheral hemorrhagic areas, erythema, edema, and necroses. They noted no differences between male and female bites.

Russell, Waldon, and Madon (1969) described 3 cases of L. unicolor Keyserling and 1 case of L. arizonica Gertsh necrotic arachnidism.

In 1967 Waldon and Russell found the first reported L. reclusa in California in San Gabriel. The spiders were found 12.2 m from stored personal belongings of a midwest family. Normally, the L. reclusa was found in southeastern and central United States from Tennessee and Alabama westward to Kansas, Oklahoma, and Texas. The first occurrence of L. laeta in North America was discovered by Levi and Spielman (1964) in 1960 in Harvard University museum building. They theorized that the L. laeta was probably introduced with a shipment of diverse materials from South America. Also, a small colony of L. laeta had been discovered in Sierra Madre, California, by Russell, Waldon and Madon (1969).

Tarantula Distribution

Spiders of the family Theraphosidae were the largest of all spiders which Americans called "tarantula." Gertsh (1949) related that a male Therophosa from Montagne la Gabrielle, French Guiana, measured 7.62 cm from front edge of chelicerae to end of abdomen and had leg span when fully extended of 25.4 cm and weighed 56.7 g. He further related that an enormous female Lasiodora, from Manaos, Brazil, had a 8.89 cm body, 24.13 cm leg span and weight of 85 g.

Snetsinger (1969), Savory (1928) and McKeown (1952) indicated that "tarantula" was a word commonly misapplied to almost any large spider. They stated that in southern Europe the "tarantula" was a sp. of wolf spider, Lycosa tarantula (L.). The poison of this European tarantula, which was named for the town of Tarentum in southern Italy, was considered by the people to be exceedingly deadly, according to McKeown (1952). Popular tradition held that the only way for the person bitten

to avoid falling into a state of coma followed by death was to indulge in dancing of a somewhat frantic type to a lively tune known as the Tarantella. Also, Snetsinger (1969) observed that many people called 1 of the giant crab spiders of the family Heteropodida a tarantula, because of its large size. This was really the banana spider, Heteropoda ventoria. The matter is further complicated by the fact that the generic name Tarantula is used for a genus of tailless whip-scorpions like the one pictured on the cover of the August, 1970, issue of the Journal of Economic Entomology.

Baerg (1958) recognized 300 spp. of spiders in the world that could be called tarantula. Minten (1959) stated that tarantulas were characterized from other spiders by fangs which moved almost parallel to each other and 2 pairs of lungs rather than 1.

In the United States about 30 spp. are distributed throughout the southwestern states, south of a line running through about the middle of Missouri, Kansas, Colorado, Utah, and Nevada, and through northern California. There are several records of their being found above this line in Utah and California. Branson (1966) states that a single sp. is known from Oklahoma, Dugesiella hentzi.

Elsewhere tarantulas were found in most parts of Mexico, throughout Central America, in most of South America, and in many islands of the Caribbean, including Trinidad, Hispaniola, Puerto Rico, and Cuba. In the eastern hemisphere they may be found in east, west, and south Africa, in Madagascar, New Guinea, Australia, India, and Ceylon.

Kaston (1953), Comstock (1948), and Baerg (1958) reported that tarantulas were nocturnal for the most part, hidden during the day inside natural cavities in the ground or under the loose bark of tree

trunks and in similar places. Some tropical species were also found in nests in trees and behind banana leaves (Baerg, 1958).

Dugesiella hentzi Life History

Baerg (1958) noted that the United States tarantulas lived in holes 45.7 cm to 60.9 cm deep which may be under stones or in the open. Baerg further stated that the tarantulas dug their holes by means of fangs aided by palpi. He observed the tarantulas in groups numbering from a few to 20 or more, living as close as .6 m apart but apparently not aware of each other. Gertsh (1949) felt that once the United States female tarantulas became attached to a burrow they stayed there during their whole life. Gertsh (1949) and Baerg (1958) observed during the winter months the tarantula opening may be plugged with silk, leaves, and soil.

Gertsh (1949) and Baerg (1958) stated it usually required 10 years for the spiderling of either sex to become sexually mature. They further noted that the immature females and males lived in similar burrows in the ground, remaining indistinguishable until the last molt.

Baerg (1958) observed a male D. hentzi migration from the middle of May to late June. He also observed another migration around Oct. 1 in Arkansas which was the mating migration. During the second migration all males traveled in the same direction. He noted that they were most often seen moving in the morning and late afternoon.

Baerg (1958) determined that the male tarantula molted for the last time when he matured while the female continued to molt annually as long as she lived up to 20 years.

Baerg (1958) conducted a test on tarantulas to determine their ability to survive with water alone and with food alone. He had 1 female survive on water alone for 2 years, 1 month, 19 days. Another female survived on water alone for 2 years, 4 months, and 6 days. On food alone he had 1 female live 1 year, 7 months, and 18 days. He concluded that in their 45.72 cm to 51.8 cm holes they probably do not drink water.

Tarantula Natural Enemies

Baerg (1958) reported that only about 20% of the tarantulas reach adulthood, and tarantulas through cannibalism being probably their own most important enemy. Gertsh (1949) listed other natural enemies as rodents, birds, lizards, frogs, toads, snakes, and Pepsis wasp. Petrunkevitch (1926) and Passmore (1936) described the battles of Pepsis wasp and tarantula for survival.

Tarantula Food

Gertsh (1949) listed tarantula food as beetles, grasshoppers, sow bugs, millipedes, other spiders, frogs, toads, mice, and lizards. He further reported 50 Sao Paulo tarantulas were kept in good health for 18 months on a diet of frogs, lizards, and snakes. Small rattlesnakes and the venomous Bothrops were killed and eaten as readily as any other kind of snake. Savory (1928) related an account of a large spider in South America which had captured and killed a pair of small birds. Baerg (1958) expressed the thought that the dominant diet of the Arkansas tarantulas in their natural habitat probably was beetles. He also thought that beetles apparently furnish substances essential to D. hentzi reproduction.

Human Tarantula Bite Cases

Thorp and Woodson (1945) pointed out a report by Browning in 1901 of 5 humans bitten by tarantulas in southern California. In 1922 Baerg (1958) induced a large female tarantula Eurypelma steindachneri to bite him twice. He experienced considerable pain at first, which disappeared in 2 hr. Of the 9 spp. (including D. hentzi) that he induced to bite him, only the Sericopolma communis from old Panama (Banana spider) was regarded as definitely poisonous to man. He reported the S. communis bites, although somewhat severe, were local not general in effect. Ewing (1928) forced fangs from Eurypelma california into his skin and squeezed the venom from an extracted venom gland into the wound. He experienced initial pain which disappeared after 4 hr. Thorp and Woodson (1945) reported 3 cases of tarantula bites with the pain of 1 bite being described as that of approximately 2 or 3 honey bee stings. Thorp and Woodson (1945), Stahnke and Johnson (1967), Minten (1959), Gertsh (1949), and Baerg (1958) concluded that although the initial tarantula bite may be painful, all United Stated spp. of tarantula were harmless to man.

Tarantula Test Animal Toxicity

Stahnke and Johnson (1967) found that a 10 mg subcutaneous injection of Aphonopelma sp. tarantula venom into the right groin of an adult rat produced, within 20 min, severe convulsions, and death in about an hr. Their post-mortem examinations revealed gastric hyperdistention, hepatic and nephric hyperemia, and pectechiae on the lungs. They pointed out that these symptoms were very similar to that evoked by the scorpion Centruroides sculpturatus Ewing.

Grothaus (1967) estimated the LD_{50} of Arkansas tarantula venom injections to white mice to be between 100 and 300 mg/kg.

Baerg (1958) tested 26 different spp. of tarantula from all over the world. He made a total of 86 tests on white rats, guinea pigs, and white rabbits. Most of his tests were made by inducing tarantulas to bite; however, in a few tests he used syringe injected venom. He reported 2/6 of the bitten rats died while 2/3 of the injected rats died when he used Dugesiella hentzi.

Venom Analysis

Spider venoms have been analyzed several ways in order to find out as much as possible about the toxic activity and composition. A tarantula (Aphonopelma rusticum) venom was analyzed by disc electrophoresis by Stahnke and Johnson (1967). They found a similarity between tarantula venom and scorpion (Centruroides sculpturatus) venom protein fractions. They further reported that enzyme analysis indicated that both venoms were L-amino acid oxidase and RNAase negative, and DNAase and protease positive. In preliminary tests they found that a guinea pig hypersensitized with scorpion venom could be triggered into anaphylaxis by tarantula venom. Nazhat (1968) found 5 protein components in hemolymph of Dugesiella hentzi using a disc electrophoresis and no protein bands of the venom.

Protein components had been found in Loxosceles venom. Using disc separation, Nazhat (1968) observed 7 distinct protein components with 3 biologically active on the American cockroach. Smith and Micks (1968) studied L. laeta, L. reclusa, and L. rufescens venom. They determined that the venom of the 3 spp. gave at least 3 distinct precipitation

lines in immunodiffusion plates. All 3 components of L. rufescens and L. reclusa appeared to be identical. Immunoelectrophoretic studies by Smith and Micks (1968) revealed the presence of 4 antigenic components in all 3 spp. However, they pointed out that venom of each species produced a distinctly different pattern.

Species of the Latrodectus genus had been studied by several authors for protein components in their venom. Muic, Stannic, and Meniga (1956) detected 6 protein constituents and determined the approximate 8.2 pH of L. tredecimguttatus. Using column electrophoresis on cellulose powder Frontali and Grasso (1964) noted 5 protein fractions with fractions 1, 2, and 4 toxic. They showed that peak 1 caused quick paralysis in the housefly; peak 2 produced symptoms in the guinea pig; and peak 4 produced slow paralysis in the housefly. Disc electrophoresis by McCrone and Netzloff (1965) exhibited 8 major fractions from L. mactans mactans, L. variolus, and L. bishopi venom but 6 from L. geometricus venom. Vicari, Bettini, Collotti, and Frontali (1965) tested 3 protein fractions of L. m. tredecimguttatus in vitro and in vivo. McCrone and Hatala (1967) isolated 7 protein and 3 non-protein fractions by gel electrophoresis. They found that only fraction B was lethal to mice. This fraction showed a 20-fold increase in lethality over that of whole venom. By using a light-scattering technique they determined that fraction B had a molecular weight of 5000 ± 1000.

Peterson (1970), using a Gilford UV Scanner at 280 lambda, showed from 2 to 3 peaks on 4 different separations using polyacrylamide gels. He also observed Lycosa sp. spider venom activity on human blood.

Weiner (1963) postulated that, by using electrophoretic separation, the toxic and lethal effects of the venom of Atrax robustus Cambridge were due to the combined action of a number of non-antigenic substances.

Fischer and Bohn (1957) used paper electrophoresis on Lycosa erythrognatha Lucas and Phoneutria fera Perty. They observed 6 protein fractions in L. erythrognatha venom and 7 protein fractions in P. fera venom. They detected no histamine or free amino acids except lysine in the venoms. They noted that the concentration of phosphate ions was exceptionally low.

Methods of Venom Recovery

Most of the studies listed in this review used some method of extracting the venom gland or part of the spider containing the gland and macerating it, thus killing the spider. Some authors have allowed the spider to bite the test animal directly. However, Shulov, Ickowicz, and Pener-Solomon (1962) recognized that there was no way of knowing the exact amount of venom injected. Lebez (1954) allowed the spider to bite a cotton pad and found that venom collected this way was more toxic than macerated venom glands.

Baerg (1958) was 1 of the first to collect venom or "milk" a spider by placing electrodes at the basal segment of the chelicerae. Denny, Dillaha, and Morgan (1964) collected spider venom from the fangs using capillary tubes after electrical stimulation. Grothaus and Howell (1967) reported a new technique for spider venom recovery. Their spiders were restrained in soft expanded plastic cells. Venoms were collected by allowing the spider to bite a small piece of folded cigarette paper when the leg was touched with a pair of electrodes from a sine wave generator. The greatest venom yield with the least

mortality was achieved with approximately 6-7 v at 15-20 cps. The venom was washed from the paper pad with distilled water.

Arachnidism Outside the United States

In Chile Donoso-Barros (1948) reported 32 cases of spider bite with Loxosceles laeta and Lycosa murina Nicolet listed as the causative agents of cutaneous gangrenous accidents. He reported 1 fatal case in an adult. He also found that a third sp. of spider, Scytodes globula, produces benign exanthematic local reactions.

Bücherl (1956) and Minten (1959) characterized a South American wandering spider Phoneutra fera as potentially dangerous. The spider was described as a large, solitary sp. that constructed no web and apparently had no permanent home. Minten (1959) related that several 100 cases of P. fera bite were reported annually in the state of Sao Paulo, Brazil. He further stated that some deaths were reported annually in children under 6 years of age. The bite caused swelling, great pain and, in severe cases, weakness and irregularity of the heart, difficulty in breathing and temporary blindness. Minten (1959) asserted that in the southern United States there were several spp. of wandering spiders belonging to a closely related genus (Ctenus) which could be poisonous.

Macchiavello (1937) first described the "gangrenous spot" or cutaneous arachnidism of L. laeta in Chile. In later publications Macchiavello (1947-48), Shulov, Ickowicz, and Pener-Solomon (1962), and Vukusic (1962) further examined the L. laeta venom. Vukusic (1962) pointed out a case of an 8 year old girl from the Province of Colchaque, Chile, where death occurred 36 hr after the girl was bitten by

Loxosceles laeta on the arm. Shulov, Ickowicz and Pener-Solomon reported a closely related sp. L. rufescens as poisonous to mice and fairly common in houses and cellars of the Mediterranean region of Israel.

Maretic (1967) found the east African orthograth spider of the genus Pterinochilus, sp. undetermined, to be venomous. Also, Finlayson (1956) in South Africa recognized the venomous activity of Latrodectus indistinctus, L. geometricus, and 4 spp. of Harpactirella including H. lightfooti.

Venom from the South American spiders, Ctenus ferus, C. nigriventer, were described by Atkins, Wingo, Sodeman, and Flynn (1958) to affect the nervous system while Nephila cruenta and Lycosa raptoria venom caused only local action.

Wallace and Sticka (1956) recognized 2 poisonous Australian spiders Latrodectus hasseltii Thorell and Atrax robustus that were greatly feared. Wiener (1957) evaluated the toxicity of A. robustus venom.

Related Venom Studies

Interesting information had been obtained for venomous animals other than spiders which sheds light on the general picture of how venoms work. Minten determined that the toxicity of rattlesnake and copperhead venom reached a peak when snakes were 6-9 months old after which there was a slight decrease. He found the most toxic rattlesnake venom sample was from a 6-9 month old snake and approximately 26 times as toxic as venom from the mother snake. Kamon (1965) used scorpion venom labelled with I^{131} to determine its movement in locusts. He

discovered that a significant part of the venom was absorbed by the pericardial cells. With time he observed that most tissues showed a decline in radioactivity except cuticular parts of the body where per cent radioactivity remained constant from 5 min to 216 hr. Schöttler (1954) established that the LD_{50} was the same for subcutaneous and intravenous venom injection of 2 Brazilian scorpions.

Benton, Heckman, and Morse (1966) reported that ambient temperature, humidity and time of injection all have a pronounced effect on the toxicity of honey bee and cobra venom in white mice and guinea pigs. They found that an initial temperature drop after injection was essential for survival of white mice and thus appeared as a possible mechanism used by the animal for detoxification of venoms. The degree of drop was dependent on the initial dosage and environmental conditions.

Carmicheal (1940) noted that the minimum fatal dose for rats kept at the lower temperature range was about 2 to 3 times the minimum fatal dose for those animals that were kept at the higher temperature range. Stahnke (1965) subjected animals to high and low temperature stress to determine the effect of stress on toxicity of scorpion and rattlesnake venom. He reported that all stressed animals were less refractory than unstressed animals. He felt that the apparent change in toxicity of the venoms seemed to be due to the physiological effects of stress rather than the temperature per se.

MATERIALS AND METHODS

Test Organisms

Test organisms used in this study included 2 insects and 2 other test animals. Test insects used were the American cockroach, Periplaneta americana (L.), and the June beetle [= Polyphaga] Lachnosterna crassissima Blanch adults. The American cockroaches were obtained from a colony maintained at Oklahoma State University. Only mature male roaches as near the same size as possible were used. The June beetles were obtained at night from around lights in Oklahoma State University married student housing. Beetles used were all collected on the same night during a 2 hr period. That night was the first night of the year that June beetles were observed by the author around lights.

Test animals used were white rabbits and white mice. The white rabbits were given to the author and included 2 females and 1 male. The mice used were Charles Rivers outbred mice reared by the Charles Rivers Mice Farm, Inc. Outbred mice were chosen for their vigor. Ten gram male mice were ordered to reduce possible variations attributable to weight and sex. However, the mice received and used had a weight variation from 8 to 12 g.

The rabbits were maintained in Biochemistry departmental rabbit cages. The mice were maintained in special cages of plastic with a wire top containing their food and water.

Laboratory Materials

Laboratory Requirements of D. hentzi. Specimens were kept in separate containers to prevent cannibalism. Containers were either round or square 1.89 l plastic freezer cartons. Round cartons proved more satisfactory since they were sturdier and required less room to store. Each container had 2 cm of sand in the bottom to make it easier to clean. A medicine cup of water was placed in each container to provide the spider a continuous supply of water. A continuous water supply was necessary to insure the best possible venom yield each week.

Spiders were individually identified as to where and when they were collected as well as their sex. This information along with venom collection and molting information was recorded and kept on a piece of tape on each container. Spiders were fed 2 to 4 American cockroach adults once a week. They were also watered at least once a week.

Spider Restrainers. The restrainers used were similar to those described by Grothaus and Howell (1967). An expanded plastic cell approximately 10 cm by 8 cm by 4 cm with an area approximately 5 cm by 3 cm and 4 cm by 2 cm cut out with scissors to allow room for the spider (Figure 5). A piece of plastic petri dish cut to the desired size was used to hold the ventral side of the tarantula. A string was wrapped around the expanded plastic cell and the plastic petri dish piece to hold the spider firmly in place. When properly restrained, the spider had its chelicerae free, but the rest of the body was restrained to allow handling.

Tarantula Recovery Equipment. Tarantula "milking" or the process of venom release and recovery was accomplished with a shock stimulus from a sine wave generator (Heathkit model IG-72) with a positive and a

negative electrode applied to the bases of the chelicerae. Small clear plastic tubes about 2 cm long with an inner diameter of 1.5 mm were placed on the fangs to collect venom (Figure 6). A 10 to 25 µl syringe was used to remove and measure the venom from the clear plastic tubes.

Electrophoresis Equipment. Tarantula venom, stomach contents, and hemolymph were electrophoresed using an Hoefer Electrophoresis Unit (model DE-102) to separate the protein fractions. An Hoefer Destainer (model DE-105) with a petroleum grade charcoal filter was used to destain gels until the fall of 1969. After the fall of 1969, a new destainer made by the physics and chemistry instrument shop at Oklahoma State University was used.

pH Paper. Broad range Dual-Tint pH paper with a 1 to 12 pH reading range was used. This paper was made by J. T. Baker Chemical Company. Two different color codes were incorporated in this paper with color comparisons at pH's of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

The narrow range pHydrion papers used were made by Micro Essential Laboratory Incorporated. A narrow range paper from 4.8 to 6.7 pH with color comparison readings at 4.8, 5.3, 5.9, 6.3, and 6.7 was used. Color comparison readings for the 5.2 to 6.6 pH paper were 5.2, 5.7, 6.0, 6.2, and 6.6. The next range of color comparisons went from 6.0 to 8.0 with 6.0, 6.4, 6.8, 7.0, 7.2, 7.6, and 8.0 readings on the color chart. The highest narrow range of pH paper needed was from 8.0 to 9.5 with 8.0, 8.5, 9.0, and 9.5 readings on the color chart.

Test Procedures

Field Collection of Spiders. After finding and identifying the tarantula entrance holes a variety of different methods were used with

varied success to capture the spiders. Pouring water down the burrows was first tried which worked to bring the spider to its entrance about 5% of the time. Usually a small stick or wire was poked down the burrow to encourage the spider's exit and to find which way the J-shaped burrow turned. If the tarantula did not exit, a spade full of dirt was removed on the side of the hole opposite of the way the burrow turned. The wire or stick was left in the hole to allow it to be followed after the spade of dirt was removed. Poking a stick further down the hole once the spade full of dirt was removed often brought the tarantula out. If all else failed, the tarantula had to be dug up taking care not to kill the spider.

Spiders were easier to collect in the morning because they often crawled out when coaxed. All spiders collected in the afternoon had to be shaded or the heat would kill them.

The spiders were allowed to crawl into .473 l plastic containers. Many of the males caught during the spring and fall migrations (term used by Baerg, 1958) were collected and brought to the laboratory by numerous people who were aware of this study. Stillwater was considered to be their locality unless it was known otherwise.

Venom Recovery. Carbon dioxide was used to anesthetize the tarantulas in their 1.89 l containers so they could be restrained for "milking." Care was taken to give the tarantula just enough CO_2 to restrain it. When this was done, venom was most likely to be obtained. The spider was teased with the "milking" tubes until it responded by biting down on the tubes. When the tarantula had demonstrated it was ready to be "milked," a small amount of a mixture of salt water and detergent was placed between the chelicerae with a medicine dropper.

This was done to allow the electrodes to make good contact. "Milking" tubes were put on the fangs and the fangs were bent down to prevent the fang tips from being broken. The sine wave generator was set at 6 v and 100 cps. Another person then placed the electrodes between the chelicerae where the salt solution had been placed. If venom was not collected, the voltage was increased to 8 or 10 v while the electrodes were still in contact with the tarantula. Care was again taken not to use too much voltage and break the fang tips. Venom that was not immediately used was placed in vials on crushed ice.

The fang tips were protected for 2 reasons. First, if the tip was broken, the venom would be contaminated with hemolymph. Secondly, tarantulas with a broken fang tip would not give any venom or very little until after their next molt.

Whole Venom Injection. The injection sites of the white mice used were trimmed of hair with scissors in preparation for inoculation. Mice used were weighed and their tails color coded for later identification. Venom was collected as previously described. After the venom was collected it was extracted from the "milking" tubes and measured with a 50 µl syringe. To prevent individual spider variation the venom was pooled in a sterilized 1 dr vial in crushed ice.

Twenty mice were injected subcutaneously in the hip with whole tarantula venom at each of 8 levels. The 8 levels of venom in µl/10 g of mouse body weight were 1, 2, 3, 4, 5, 6, 7, and 9 µl/10 g. Also, 10 mice were each injected with 9 µl of venom intracutaneously in the hip. Nine µl of venom were each injected into 10 mice subcutaneously in the chest. Nine µl of venom were each injected intramuscularly into the hip of 10 mice. Finally, 3 µl of venom were each injected

intravenously into the right femoral vein of 10 mice. The intravenous injections were made with a 26 gauge needle and 5 µl syringe by clipping the skin over the vein thus exposing it for injection. All other injections were made with a 26 gauge needle and a 50 µl syringe. Great care was taken to prevent injecting air bubbles.

Mice which died after venom injections were immediately cut open along a mid-ventral line with scissors. All internal organs including the heart, brain, lungs, kidneys, and intestines were removed and placed in a jar of buffered formalin. Also, skin and skeletal muscle at the injection site were taken and were placed in the jar of formalin. One jar was used for each mouse and appropriately identified. Tissue slides were made by the Veterinary Pathology Department at Oklahoma State University. One slide was made per dead mouse which included sections of the heart, lungs, adrenal glands, brain, kidneys, intestines, liver, skin, and skeletal muscle. These slides were examined by Dr. B. C. Ward of the Veterinary Pathology Department for histologic abnormalities.

The right hips of the 3 white rabbits used were trimmed of hair with electric clippers in preparation for inoculation. A new, presterilized 1 cc tuberculin syringe and 26 gauge needle was used for each inoculation, then discarded. Measured venom was then placed in the tuberculin syringe set at 0.1 cc and already full to 0.07 cc with 0.09% saline. Then 0.09% saline was drawn into the microsyringe and placed in the tuberculin syringe until it was full to 0.1 cc. The tuberculin syringes were prepared with venom and were put on ice until the rabbits could be inoculated.

The rabbits were hand held by 1 person while another person did the inoculating. The trimmed hip was exposed. A 26 gauge 1.27 cm needle was inserted so the venom could be deposited subcutaneously approximately 1.27 cm from the site of insertion. The injection was made and the needle quickly removed. Following injection the rabbits were returned to marked individual cages for observation. The time lapse from "milking" to inoculation was approximately 30 min. The hip was selected as the injection site since any localized response to the venom might manifest itself by affecting the walking behavior of the rabbit.

Roach LD_{50} Determination. Fifty mature American cockroach males were used at each of 6 treatment levels. Venom was collected and pooled as usual. The venom was diluted to 10% with 0.9% physiological saline. The roaches were injected with a 32 gauge needle and a 50 µl syringe. The needle was inserted through the eighth abdominal intersegmental membrane and run up under the exoskeleton to the second sternal plate where the desired amount of saline and venom mixture was deposited. The needle was quickly removed to prevent any loss of injected material.

One, 2, 3, 4, 5, and 6 µl of the 10% venom in saline mixture was injected into 50 roaches each. Thus, .1, .2, .3, .4, .5, and .6 µl of actual venom were injected into each of the 50 roaches at each level.

Electrophoresis. Acid cleaned 12.7 cm columnar tubes were used for electrophoresis. These tubes were precoated with a column coater, to permit gel removal. The tubes were stoppered and placed stopper down in a filling rack. Stock solutions A and C were added to a flask in equal volumes. Ammonium persulfate was added to the mixture and

stirred in. The columnar tubes were filled to a premarked level with this solution. To prevent a meniscus formation, a small amount of water was placed on top of each tube. The tubes then stood for 30 min in total darkness. After 30 min, the water was drawn off each tube and buffer 10x added. The buffer contained 3.0 g of Tris. and 14.4 g of glycerine distilled water added to bring the total volume to 1 liter. The stoppers were removed before the columnar tubes were placed in the electrophoresis unit. The lower part of the unit was filled with buffer 10x at a pH of 8.33 to a set level. The upper part received 400 ml of buffer 10x at a pH of 8.34. The loaded electrophoresis unit was run for 30 min to eliminate any residual catalyst. A measured amount of venom, hemolymph, or stomach contents was layered on top of each gel. Electrophoresis was conducted at 5 mA per sample tube for 1 hr. After the gels were removed, they were stained with 0.5% aniline black 7% acetic acid for 3 hr. Excess dye was removed with a destainer containing 7% acetic acid electrical current. Upon completion of destaining, the gels were placed in a stoppered test tube with 7% acetic acid.

A slightly different electrophoresis technique was used for some of the gels. These gels will be referred to as the small gels. The procedure used was according to Carralco Instruction manual for 7% standard acrylamide gel using both stacking and separating gels. The previous technique did not use a stacking gel to make the fractions more compact. Gels were poured into glass tubes with an inner diameter of 2.8 mm and 10 cm long. Thus, each of these gels contained 1/3 the amount of separating gel previously used. Depth of the stacking gel was approximately 1.5 cm while the depth of the separating gel was

approximately 6.5 cm. A 20 µl sample with tracking dye was layered on each prepared gel in 5% sucrose which also contained buffer (Tris.-glycine, pH 8.5) at the same concentration as in the upper buffer. Electrophoresis was stopped when the tracing dye was approximately 1 cm from the bottom of the tube.

Protein Fraction Injection. Mature American male cockroaches were used to test protein fraction toxicity. Venom was collected by the method previously described. The venom was combined, and 5 µl placed on each of at least 2 gels. Following electrophoresis, 1 gel was stained and at least 1 gel left unstained. To prevent any possible loss of toxicity the unstained gel was immediately wrapped in aluminum foil and placed in a freezer until used. Only 1 gel was stained, for the staining procedure denatures proteins. Following staining and destaining the stained gel was matched up beside an unstained gel. The unstained gel was then sectioned with a razor blade where desired (Figure 12). Each sectioned portion of gel was put in a test tube containing 0.4 ml of 0.9% saline. The gel was broken up and mixed thoroughly with the saline by a glass stirring rod. The mixture was allowed to stand for 15 min at room temperature. To separate the gel from the saline containing the protein, the mixture was centrifuged for 15 min. Approximately 0.25 ml of protein and saline was drawn off the top of the settled gel with a small pipette. The mixture was put in 1 dr vials and placed on ice.

Two healthy, mature male American cockroaches per treatment were selected and placed in a .237 l ice cream carton with a clear plastic lid. A 0.5 cc syringe with a 26 gauge needle was used to inject the roaches. The needle was inserted through the eighth ventral

intersegmental membrane and run carefully up under the exoskeleton to approximately the second abdominal segment. The roach was then injected with 0.1 ml saline and protein mixture. Care was taken to remove the needle so none of the mixture would come out of the insertion hole. The injected roaches were kept in the cartons for observation at 80 F ± 3.

Smaller gels were used for the April 22, 1970, roach and June beetle studies. Three µl of venom were applied to each of 8 gels. Thus, 7 gels were sliced and the segments of all 7 mixed with 1 ml of 0.9% saline. Approximately 0.8 ml extract was drawn off the gel. The June beetle injections were made similar to the cockroach injections.

Mice were injected with protein fractions prepared like the small gels used in the June beetle study. Seven gels each had 5 µl of venom applied. Preparation of the saline and protein mixture was the same as in the June beetle study. Each mouse was injected with 0.3 cc of saline and protein mixture.

Red Blood Cell Hemolysis. Human red blood cells were washed by placing 1 cc of fresh whole blood and 3 cc of 0.9% saline in a plastic centrifuge tube. This tube was centrifuged at 10,000 rpm at 0 C for 15 min. The supernate was poured off. Then 3 cc of saline were added to each tube and the cells were gently resuspended in the saline. This washing process was repeated 4 times.

These washed red blood cells were suspended in 2 cc of saline. Twenty µl of venom were added to this mixture. Two tubes were used as control. Once the venom was added, the tube was allowed to sit in crushed ice for 30 min then centrifuged for 10 min at 10,000 rpm and 0 C. The extent of helolysis was then observed and recorded.

Fresh whole human blood on a microscope slide was also used to determine a low level of venom hemolytic activity. A cover slide was placed on top of the blood. A measured amount of venom was added with a 10 µl syringe alongside the cover slip. The result was viewed under a compound microscope.

Determination of pH. For tarantula pH determination the spiders were "milked" by the method previously described. The venom was taken out of the "milking" tubes for each spider with a 25 µl syringe. Broad range pH paper was spotted first, with the reverse side of the paper used for comparison with the color scale.

Other spiders used, which were "milked" under a binocular microscope, included the Latrodectus mactans, Loxosceles reclusa, and Agelenopsis naevia (Walckenaer). Electrical shock was not used on the spiders unless necessary. When electrical shock was used, a setting of 2 v and 20 cps was best. Higher voltage caused the spiders to expel stomach fluid. An electrical shock was needed with the Lycosa rabida to obtain venom. For these spiders the generator was set at 4 v and 20 cps. Best results were obtained when the shock was placed on the membrane at the base of the chelicerae.

A small piece of .63 cm square, broad range paper was tested first followed by narrow pH. The paper was held with forceps between the chelicerae where the fangs fold back into the fang furrow. The 4 smaller spiders were enticed to bite the pH paper and release their venom. When a venom spot was obtained on the pH paper, it was compared with the color scale for the range used.

RESULTS AND DISCUSSION

Field Collection Observations

One of the biggest problems in this study was to learn enough about the ecology of *D. hentzi* in Oklahoma to collect an adequate number of spiders for venom studies. After searching much of the area around Stillwater, the first tarantula other than roving tarantulas of "milkable" size was found 2 miles west of Pawnee in a burrow. Much was learned about the ecology of the tarantula from the 239 specimens collected that would aid any future efforts to collect this spider.

The best places found to begin looking for tarantulas were areas where male tarantulas were regularly seen crossing the highway. Two male migrations were observed in Oklahoma, as noted by males crossing the highways. During the early summer migration, males were caught beginning May 24 and ending July 27. During the fall migration males were captured from Sept. 12 to Sept. 30. After locating spots where males were seen on the highway, the search for tarantula burrows would radiate from there. Often an old pond dam would be a good place to start looking.

Most hilly pastures in an area where males were seen on the highway produced some tarantulas. Approximately half way down hills on the edges of small eroded bluffs or lightly vegetated areas were good places to find tarantula burrows. Where the pasture grasses were tall, the tarantula entrance holes, although probably there, were extremely difficult to see.

Two immatures approximately 2 years old and 1 year old were found separately in shallow burrows under rocks. The age could be estimated since immature tarantulas have a large black abdominal spot (Figure 3) for approximately the first 7 years of their lives. Only after the tarantulas had lost this spot were they found to dig their holes in an open area. Apparently some form of habitat protection is needed for survival during the first years of the tarantula's life.

According to Baerg (1958) female tarantulas were fertilized in the fall during the fall male migration. If this is true, apparently the females carry the male sperm nearly 1 year before eggs are deposited. Females captured were found to have laid their eggs in the laboratory from June 19 to July 7, which is before the males' migration starts in Sept.

It is interesting to note that tarantulas were never collected in forested areas. If they were found among trees, it was always in a grassy clearing. Although Baerg had reported that many tarantulas found in Arkansas were under rocks or in holes under rocks, only 4 immatures were found under rocks around Stillwater.

Usually at least 1 tarantula was found in a pond dam if any were found in the area. An immature was collected in a cement basement after the cellar door was left open overnight. When males were migrating, they were found in barns, sheds, under boards, and in screened porches.

Most immature tarantulas and mature female tarantulas were found in the ground in burrows. These burrows usually went straight down for 25.4 cm and over horizontally 10.2 to 15.3 cm with a 5 to 10 cm vertical drop while going the horizontal distance. Some tarantulas have chambers about 8 to 9 cm across (Figure 4). Since those tarantulas

with chambers were always large females, many with egg sacs, the chambers were probably for egg laying. Sometimes a shallow hole about 10 cm long branched off the main burrow and contained a molted tarantula skin. This was usually in burrows containing young spiders which weighed 4 to 5 g. Entrance holes for immatures were 1.27 cm to 1.91 cm across. The diam of mature female holes was from 2.54 to 5.08 cm across with most 2.54 to 3.18 cm across. One burrow went down 33 cm before curving then over 79 cm horizontally while dropping 23 cm. The total depth of this hole was 56 cm (Figure 4). No mature male tarantulas were ever found in burrows after June 10. Thus, it might be assumed that males are free ranging until their death probably during the winter.

Ten tarantula holes were dusted with orange Dayglo.[1] The Dayglo was applied in a 4 in diam circle around the entrance hole. The dusted holes were checked at midnight with a black light and at 9 o'clock in the morning. All but 2 left trails no further than 1.26 m from the hole. Two tarantulas ranged to 3.7 m and 4.3 m from their holes, stopping at dried cow paddies and apparently checking under and around them for food. Two Lycosa holes were accidentally dusted. These 2 Lycosa spiders left trails out to 4.6 and 4.9 m from their holes also wandering around from cow paddy to cow paddy.

Insect exoskeletons left in and around tarantula holes were examined. Most of the exoskeletons were either Scarabidae or Carabidae but 1 Blattidae exoskeleton was found. Probably the main diet of the

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[1]Made by Day-Glo Color Corporation.

tarantulas around Stillwater consisted of dung beetles since their exoskeletons and their dung balls were found most often around the holes.

Laboratory Observations

Several observations were made of the differences between the sexes of the tarantulas while they were held in laboratory captivity. First, mature males needed to eat more often than did the females. Also, males needed water available at all times while females may go for a month without water with no ill effects other than a lowered venom yield. Males were observed to molt in the laboratory from June 1 to June 12. Prior to this molt they were captured in burrows and were indistinguishable from the females. June 1 coincides closely with the beginning of the male early summer migration. Thus, it could be assumed that the immature males molt into visible mature males then leave their holes and migrate. Since males live in the same area as the females until maturity, apparently their movement in mass of any distance could be a means by which they reduce inbreeding.

A behavioral characteristic observed in the laboratory may help distinguish between immature males and females which externally look alike. In the laboratory only females were observed to wrap insect debris in webbing. Mature males seemed to spin no webbing except a spermweb and left their debris where it fell. This differential behavioral characteristic was observed year round. In observing immatures about half wrapped their debris in webbing while half left their debris lying around.

Four spiders with broken fangs and one without palps were observed

to have the missing parts restored during the next molt. The palps replaced were reduced in size while the broken fangs replaced were usually normal in size.

Table 1. Observations on D. hentzi males in the laboratory.

Average wt. when caught	5.54 grams
Average first month weight loss	.42 grams
Average time kept in captivity before death	88.7 days
Dates molting to male	June 1 to June 12
Range of dates males collected in summer	May 24 to July 27
Range of dates males collected in fall	Sept. 12 to Sept. 30

Table 2. Observations on D. hentzi females in the laboratory.

Range of dates females laid egg sacs in laboratory	June 19 to July 7
Average weight of females laying eggs	8.73 grams
Range of weights of females laying egg sacs	4.76-14.38 grams
Average weight of egg sac	4.00 grams
First observed spiderling hatch	August 5, 1970

In reviewing the laboratory records kept on the tarantula, some general observations were made. Of the 23 males collected during 1969, only 3 or 13% survived through the winter to June 1, 1970. Thirty-three

percent of the females collected during 1969 survived to June 1, 1970. Two out of 6 males collected from burrows in the spring of 1970 died while molting. The 2 spiders which died had shed all their exoskeleton except the chelicerae. This was also observed in several females. Apparently the last molt to a mature male and later female molts are critical for the spider's survival. The largest male weighed was 10.20 g, and the largest female weighed was 17.54 g. However, the average male weight was 5.54 g. The most venom collected from a male was 11 µl while 1 female gave 34 µl of venom.

After spiders reached 3 g with an average monthly weight gain of 1.49 g as shown in Table 3, there was a general decrease in monthly weight gain up through 17.6 g spiders. The biggest growth spurt took

Table 3. D. hentzi "milking" data.

Tarantula wt. range in g	Sex	Avg. wt. gain/ mo.	No. tarantulas "milked"	Avg. times each tarantula "milked"	Avg. µl/ "milking"/ tarantula
5.54 (Avg.)	M	-.42	23	2	3.7
2.00-2.99	I[a]	.39	--	-	--
3.00-3.99	I[a]	1.49	2	2.5	2.6
4.00-4.99	F	1.03	20	2.8	5.0
5.00-5.99	F	.75	16	2.3	6.9
6.00-6.99	F	.71	14	2.4	8.4
7.00-7.99	F	.74	13	3.7	9.5
8.00-8.99	F	.43	11	3.1	8.7
9.00-9.99	F	.05	11	4.0	9.2
10.00-10.99	F	-.49	14	4.8	7.5
11.00-11.99	F	-.28	7	5.0	5.3
12.00-12.99	F	-.17	6	3.1	12.1
13.00-17.6	F	-.35	8	3.5	14.1

[a]I is immature spider.

place in the laboratory with spiders which were collected at 3 to 4.99 g weight. Spiders with the largest average venom yield were those that weighed 12 g or more when they were caught. The more often the spiders were milked the lower the venom yield as shown by 10 to 11.99 g spiders in Table 3. From individual milking records not shown on Table 3 all spiders which molted gave no venom for 2 to 3 weeks prior to their molt.

Rabbit Venom Injections

Three rabbits were injected with venom collected May 20, 1970. Each rabbit was injected with a 0.1 cc mixture of a measured volume of venom and 0.9% saline. Rabbit number 1 was given 10 µl of venom in saline. Rabbit number 2 was injected with 15 µl of venom in saline. Rabbit number 3 was injected with 20 µl of venom in saline. The rabbits were observed at 1, 6, 12, 24, 48, 72, and 96 hr. At none of the levels used was there any discoloration at the injection site nor was there any observed abnormality in the way the rabbits walked.

LD_{50} of White Mice to D. hentzi Venom

To determine relative toxicity of D. hentzi venom, an LD_{50} study was set up using 20 mice per treatment level. Although the mice were constantly checked after injection, all mice that died were dead within 6 hr. Mice that did not die were held for 72 hr then killed with CO_2.

The results of the LD_{50} study are listed in Table 4. An LD_{50} line was not drawn because of the jump in the number of dead mice from 2 µl with none dead to 3 µl with 55% dead. The equipment available to the author would not allow the accurate measurement of venom closer than to the nearest µl. Thus, there were no points below 50% to allow an

accurate determination of the LD_{50}. However, the LD_{50} level of venom for white mice between 3 µl per 10 g body weight and 2 µl per 10 g body weight was probably around 2.8 µl of venom per 10 µl of body weight.

Table 4. LD_{50} of white mice when injected subcutaneously in the hip with D. hentzi venom.

Injection rate in µl/10 g body weight	At 6 hr	
	No. of dead mice out of 20	Per Cent Dead
1	0	0
2	0	0
3	11	55
4	13	65
5	15	75
6	19	95
7	20	100
9	20	100

Mice Post-Mortem Pathologic Examination

As soon as a mouse died or was killed with CO_2, it was cut open and the internal organs removed. The organs removed were fixed in 10% formalin and stained with hematoxylin and eosin. Sections were made of the skin, skeletal muscle, kidneys, adrenal glands, heart, lungs, intestine, liver, and brain.

Upon microscopic examination of the prepared tissue section slides, 2 types of histologic lesions were observed by Dr. B. C. Ward. The first type of lesions observed was acute lesions (Figure 7) which

were observable within the first few hours after injection. These lesions were found in the skeletal muscle (Figure 8) near the injection site and in the cardiac muscle. The lesions in the skeletal muscle were swelling of the muscle fibers and loss of cross striations (called Zenker's necrosis), separation of muscle bundles and subcutaneous edema. In the heart the affected muscle fibers were found in small groups and appeared more acidophilic than the surrounding normal fibers. The nuclei were small and dark (pyknotic).

The second type of lesions were subacute lesions found in mice which died or were sacrificed 72 hr after injection. They were seen in the same sites as the acute lesions. The muscle fibers at the injection site showed advanced stages of necrosis (Figure 9). The muscle cells were broken up and disoriented. The nuclei were very pyknotic. Very basophilic granular material (probably calcium) was deposited on the degenerating muscle. Inflammatory cells had infiltrated the injection site and edema was pronounced. The changes in the heart which probably caused death were similar except there were no inflammatory cells or edema (Figure 10). The mineral deposition was more pronounced, however.

A skin lesion was observed in one mouse (Figure 7). The skin lesions consisted of acute necrosis of the epithelium. The area of skin necrosis was limited to a 3 mm radius around the injection site. The small area of necrosis probably explains why necrosis was not seen in other slides if the tissue sections were not taken from this small area. Severity of lesions depended mostly on the time after injection the mouse lived and somewhat upon the quantity of venom.

Mice Injection Site Comparisons

Since the destruction of muscle cells in the heart was the most probable cause of death, it was theorized that the faster a lethal dose of venom reached the heart through the veins the faster a mouse would die. To determine the validity of this theory a test was set up where mice were injected intravenously, intramuscularly, subcutaneously, and intracutaneously. Intravenous injections were made into the right femoral vein. Intramuscular injects were made in the right hip. Subcutaneous injections were made in the right hip and in the chest area over the heart. Ten mice were used per treatment.

Table 5 lists the results of that test. Mice injected intravenously died in 27 to 70 seconds or a 45 second average. Mice injected intramuscularly died in 18 to 58 min or a 29 min average. Mice injected subcutaneously in the chest died in 4 to 132 min or a 46 min

Table 5. The effect of injection site of D. hentzi venom on white mice expressed as time from introduction of venom to death.

Injection site	µl venom/10 g body wt.	Range of times from injection to death	Avg. time of death after injection
Intravenous (right femoral vein)	1.5	27-70 seconds	45 seconds
Intramuscular (right hip)	9	18-58 minutes	29 minutes
Subcutaneous (chest)	9	4-132 minutes	46 minutes
Subcutaneous (right hip)	9	26-124 minutes	66 minutes
Intracutaneous	9	70-376 minutes	161 minutes

average. Mice injected subcutaneously in the right hip died in 26 to 124 min or a 66 min average. Finally, mice injected intracutaneously where the venom had to pass through skin layers died in 70-376 min or a 161 min average. This supports the theory previously mentioned that the quicker a lethal dose of venom gets to the heart through the veins the quicker death will occur.

Mice Symptoms Resulting from Venom Injection

As the result of venom injections several general symptoms were observed prior to death of the mice. First, mice injected in the hip immediately favored that hip. The injected mice then closed their eyes, sat in 1 place, and became very docile (they would not try to bite or escape when picked up). Breathing quickened. Later (time varied with injection site) the mouse stretched out and lay immobile with its injected leg extended and rigid. Finally, convulsions started. These convulsions were expressed as a series of violent muscle contractions. The mice usually died within 5 min after convulsions started. The occurrence or timing of these symptoms varied with the injection site. However, mice injected intravenously in the femoral vein started convulsions within 5 seconds post injection and died in about 45 seconds, thus skipping the first symptoms.

LD_{50} of D. hentzi Venom to Roaches

Three hundred adult male American cockroaches were injected with a 10% venom in 0.9% physiological saline mixture. Fifty roaches were injected at each level of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 µl tarantula venom. Mortality readings were taken at 2 hr, 6 hr, 48 hr, and 96 hr. The roaches were called dead when they did not move an appendage when

poked with a pencil. A 6 hr LD_{50} line sets the LD_{50} at 0.31 µl per adult male American cockroach. This line would be elevated with time as shown on Table 6 of all the results.

Table 6. LD_{50} of D. hentzi venom to American cockroaches.

µl venom	% Mortality of 50 mice after:			
	2 hr	6 hr	48 hr	96 hr
.1	0	24	30	48
.2	12	34	46	52
.3	8	48	56	68
.4	32	64	82	86
.5	18	78	90	90
.6	26	88	96	96

Protein Fraction Injections

Four separate tests were performed to learn where the toxicity of tarantula venom was located when it was run through an electrophoresis separation gel. First, male and female venom protein fractions were compared. The second test compared the toxicity of venom gel fractions to June beetles with the toxicity of venom gel fractions to American cockroaches. Also, the second test was used to compare the seasonal toxicity venom gel fractions tested in the spring against the toxicity of venom gel fractions tested in the summer. The third test was designed to try to locate a toxic area of the separation gel to white mice. The final test was designed to determine if a toxic component was present in separation gels run with the positive and negative poles reversed from normal electrophoresis procedure.

In the first test large gels were prepared for cockroach injection as previously described. Male and female tarantula venom was collected. Five µl of venom was used per gel. One gel each per male and female was sliced as shown in Figure 11. Three different replicates on 3 different days with venom from different spiders were run. The results are listed in Tables 7 and 8. Roach toxicity was observed in the top half of the gels with the peak toxicity present in the area of the second visible band. Some of the gel slices temporarily paralyzed the roaches at 24 hr observation and allowed roach recovery by the 48 hr observation. This could be due to slicing the fringe areas of the toxic fraction or a difference in the action of the fractions. Male and female fraction toxicity was essentially the same.

In Tables 7 through 11 the following abbreviations will be used:

CP -- Complete paralysis with no movement obtained by blowing on or touching the roach.

PP -- Partial paralysis with antennae or leg movements when roach touched and/or blown on, however, roach cannot walk.

SM -- Slow motion, roach can walk but all movement is noticeably slowed from normal.

N -- Normal, response to stimuli is rapid and normal.

Small gels were used for the second test and sliced as shown in Figure 13. All June beetles and American roaches received injections from the same pooled extract per treatment, thus reducing some technique error. One June beetle was injected with 2 µl of pure venom for comparison with the sliced gel results. June beetles were used since Baerg (1958) stated that beetles were the major part of a tarantula's diet. The stacking gel was prepared like the other sliced to determine if any toxic component remained in the stacking gel. Results are

Table 7. Toxicity of electrophoresed protein bands of male D. hentzi venom when injected into Periplaneta americana.

Band identification	Condition of 2 cockroaches after:			
	2 hr	5 hr	24 hr	48 hr
Replicate No. 1 (Aug. 8, 1969)				
A	2PP	2CP	2PP	2SM
B	2PP	2CP	2CP	2CP
C	2SM	2SM	2SM	2SM
D	2N	2N	2N	2N
E	2N	2N	2N	2N
Check	2N	2N	2N	2N
Replicate No. 2 (Aug. 13, 1969)				
A	2PP	2PP	2SM	2N
B	2CP	2CP	2CP	2CP
C	2SM	2SM	2SM	2SM
D	2N	2N	2N	2N
E	2N	2N	2N	2N
Check	2N	2N	2N	2N
Replicate No. 3 (Aug. 20, 1969)				
A	2PP	2CP	2CP	2CP
B	2PP	2PP	2CP	2CP
C	2SM	2SM	2N	2N
D	2N	2N	2N	2N
E	2N	2N	2N	2N
Check	2N	2N	2N	2N

Table 8. Toxicity of electrophoresed protein bands of female D. hentzi venom when injected into Periplaneta americana.

Band identification	Condition of 2 cockroaches after:			
	2 hr	5 hr	24 hr	48 hr
Replicate No. 1 (Aug. 6, 1969)				
A	1CP,1SM	1CP,1SM	1CP,1SM	1CP,1N
B	2CP	2CP	2CP	2CP
C	2SM	1CP,1SM	1CP,1SM	1CP,1N
D	2SM	2SM	2N	2N
E	2N	2N	2N	2N
Check	2N	2N	2N	2N
Replicate No. 2 (Aug. 13, 1969)				
A	1PP,1SM	1CP,1PP	1CP,1PP	2SM
B	2PP	2PP	2PP	2SM
C	2SM	2SM	2SM	2CP
D	2N	2N	2N	2N
E	2N	2N	2N	2N
Check	2N	2N	2N	2N
Replicate No. 3 (Aug. 20, 1969)				
A	2CP	2CP	2CP	2SM
B	1CP,1PP	1CP,1PP	2CP	2SM
C	2SM	2PP	1PP,1SM	2SM
D	2N	2N	2N	2N
E	2N	2N	2N	2N
Check	2N	2N	2N	2N

listed in Tables 9 and 10. Gel slices B and D exhibited toxic activity on the beetles while the stacking gel and slices A, B, and C exhibited some toxic activity on roaches. Location of the toxic component for roaches was very similar to that exhibited in the first test. The beetle injected with whole venom was dead at the 24 hr observation. Forty-eight hr after injection the beetle was examined. Everything inside the exoskeleton was found completely decomposed and only a fluid which smelled like decaying tissue remained. All other beetles were examined, and 3 of the 4 beetles in slice B and 2 of the 4 beetles in slice D were found to have similar decomposition. No decomposition was observed in the roaches. Apparently D. hentzi injects a substance in their venom which causes cell destruction in beetles while other components immediately paralyze the prey.

One month and 9 day old mouse injected with 0.3 cc of extract prepared nearly identified to that in test 2. Because of the limited amount of extract only 1 mouse was used per treatment. Mice injected with extract from slices B, C, and E were less active than the rest of the mice for the first 24 hr. After that no deviation from normal was observed. No mice were dead 14 days after the injections were made.

No references were found in the literature referring to the toxicity of spider venom run through separating gel with the electrophoresis poles reversed. The gel was sliced as in Figure 13. The results of the fourth test are listed in Table 11. Toxic activity was descending as the slices moved down the gel. Toxicity was exhibited in gel slices A, B, C and the stacking gel.

The number of visible bands present in each gel varied. This was mostly due to improved electrophoresis technique, thus gels prepared

Table 9. Toxicity of electrophoresed proteins of female D. hentzi venom[a] when injected into Lachosterna crassissma.

Gel slice identification	Condition of 4 June beetles after:		
	2 hr	5 hr	24 hr
Stacking gel	4N	4N	4N
A	4N	4N	4N
B	4N	4N	2CP,1PP,1N
C	4N	4N	4N
D	4N	4N	2PP,2N
E	4N	4N	4N
Check	4N	4N	4N

[a]Collected April 22, 1970.

Table 10. Toxicity of electrophoresed proteins of female D. hentzi venom[a] when injected into Periplaneta americana.

Gel slice identification	Condition of 2 cockroaches after:		
	2 hr	5 hr	24 hr
Stacking gel	2PP	2PP	2CP
A	2PP	2PP	2CP
B	2PP	2PP	1PP,1CP
C	2N	2N	1PP,1N
D	2N	2N	2N
E	2N	2N	2N
Check	2N	2N	2N

[a]Collected April 22, 1970.

in the spring of 1970 showed more bands than those prepared in the summer of 1969.

Table 11. Toxicity of negatively electrophoresed proteins of female D. hentzi venom[a] when injected into Periplaneta americana.

Gel slice identification	Condition of 4 cockroaches after:			
	2 hr	5 hr	24 hr	48 hr
Stacking gel	3PP,1SM	2CP,1PP,1SM	1PP,3SM	3N,1SM
A	4PP	3CP,1PP	4CP	4CP
B	2CP,1SM,1N	1CP,2PP,1SM	3CP,1SM	3CP,1SM
C	1PP,3SM	2PP,2SM	1CP,3SM	1CP,3SM
D	4N	4N	4N	4N
E	4N	4N	4N	4N
F	4N	4N	4N	4N
Check	4N	4N	4N	4N

[a]Collected June 12, 1970.

Using the large gel electrophoresis technique, Figures 14, 15, and 11 show the difference between tarantula hemolymph, stomach contents, male venom and female venom. Two different spiders were used in each of the 2 hemolymph gels and each of the 2 stomach content gels.

Spider Venom pH Determination

In reviewing the literature only 1 reference was found on the pH of spider venom. Muic, Stannic, and Meniga (1956) stated that the approximate pH of Latrodectus tredecimguttatus was 8.2. However, they did not report their method of pH determination.

Tarantula venom was tested for pH by using pH paper. It was decided to use pH paper because of the low cost and small quantities of material being tested. Tarantula venom collected June 1, 1970, was tested on broad range (1 to 12) pH paper and found to be between the 5.0 and 6.0 pH range when compared with the color code. When 4.8 to 6.3 narrow range pH paper was used, the venom spot fell between the 5.3 and 5.9 color comparison readings. Three mature males, 3 mature females, and 3 immature tarantulas were tested to determine if the sex or age of the spider made any difference in the pH of the venom. All spots on the pH paper for all 9 individual spiders used were exactly the same color. By personal communications, the author found that Dr. Floyd Schanbacher has determined the pH of tarantula venom to be 5.44 using a Fisher Accumet pH meter.

Four other spider species, which were maintained in the laboratory, were tested for their venom pH to use as a comparison with the pH of the tarantula. Venom pH determination was the primary goal with these 4 spp. However, gut fluids and hemolymph were tested when available. Because of the small size of the venom spots for these 4 spp. the spot was placed under a binocular scope at 10X magnification to match with the color chart.

Only female black widows Latrodectus mactans were available for testing. For the 3 females used the broad range pH paper indicated a neutral pH reading of 7.0. However, when a 6.0 to 8.0 narrow range pH paper was used, a 6.8 reading was obtained. The venom spots were identical in color for the 3 female spiders used.

The next spider tested was the brown spider L. reclusa. Brown spider venom was between 7.0 and 8.0 on the broad range pH paper. The

narrow range 6.0 to 8.0 paper gave a 7.6 reading. In 2 cases brown spider hemolymph was tested. The 1 female spider indicated 6.8 pH on 6.0 to 8.0 paper. A male indicated an 8.0 reading on 8.0 to 9.5 and 6.0 to 8.0 papers. However, the male's pH dropped to 6.8 for hemolymph after 15 min of losing hemolymph. Thus variation was probably due to individual differences rather than sex differences. Gut content had a pH of 6.0 on the broad range pH paper.

Agelenopsis naevia gave identical color readings for venom. Color of the venom spot matched the 6.3 pH color exactly on the 4.8 to 6.3 pH paper's color chart. The broad range pH paper indicated the venom was between 6.0 and 7.0. Although the venom was acid the gut content and hemolymph were basic. Hemolymph pH was 7.6 for 1 female on 6.0 to 8.0 pH paper and 8.0 for another female on 8.0 to 9.5 and 1 to 12 range pH papers. Gut content was between 8 and 9 on the broad range and 8.5 on the narrow range 8.0 to 9.5 paper for both a male and a female.

In testing the Lycosa rabida pure venom was easily obtained and was identical from spider to spider. Only females were available. Thus, only 3 female spiders were used. Gut content was basic between 8.0 and 8.5 pH on 8.0 to 9.5 paper. L. rabida venom matched the color chart exactly at 5.9 pH on 4.8 to 6.7 pH paper.

Apparently there is an inherited physiological system in each of the 5 spiders tested which maintains a constant pH for venom of the species. According to Comstock (1948) the venom gland is an extension of the hemolymph system in spiders. However, in the case of A. naevia the venom was acid (6.3 pH) while the hemolymph was basic (8.0 pH). The reverse was true in the L. reclusa. The hemolymph was slightly acid (6.8 pH) in one case while the venom was basic (7.6 pH).

Table 12 lists the venom and other body fluid pH determinations of D. hentzi, L. mactans, A. naevia, and L. reclusa. The abbreviations and symbols which will be used in the ensuing table are as follows:

Indiv. -- Individual
I -- Immature
F -- Female
M -- Male

Table 12. D. hentzi, Latrodectus mactans, Agelenopsis naevia, and Loxosceles reclusa venom and other body fluid pH determinations.

Indiv. no.	Spider sp.	Type body fluid	Date tested	Broad-range pH reading	Narrow-range pH reading
I-1	D. hentzi	Venom	6-1-70	5.0	5.3-5.9
I-2	D. hentzi	Venom	6-5-70	5.0	5.3-5.9
I-3	D. hentzi	Venom	6-5-70	5.0	5.3-5.9
M-4	D. hentzi	Venom	6-1-70	5.0	5.3-5.9
M-5	D. hentzi	Venom	6-5-70	5.0	5.3-5.9
M-6	D. hentzi	Venom	6-5-70	5.0	5.3-5.9
F-7	D. hentzi	Venom	6-5-70	5.0	5.3-5.9
F-8	D. hentzi	Venom	6-5-70	5.0	5.3-5.9
F-9	D. hentzi	Venom	6-5-70	5.0	5.3-5.9
F-10	L. mactans	Venom	6-2-70	7.0	6.8
F-11	L. mactans	Venom	6-2-70	7.0	6.8
F-12	L. mactans	Venom	6-2-70	7.0	6.8
F-13	L. rabida	Venom	6-2-70	6.0	5.9
F-14	L. rabida	Venom	6-2-70	6.0	5.9
F-15	L. rabida	Venom	6-2-70	6.0	5.9
F-16	L. rabida	Gut	6-2-70	8.0	8.0-8.5
F-17	A. naevia	Venom	6-2-70	6.0	6.3
F-18	A. naevia	Venom	6-2-70	6.0	6.3
M-19	A. naevia	Venom	6-2-70	6.0	6.3
M-19	A. naevia	Gut	6-2-70	8.0-9.0	8.5

Table 12 (Continued)

Indiv. no.	Spider sp.	Type body fluid	Date tested	Broad-range pH reading	Narrow-range pH reading
F-20	A. naevia	Gut	6-2-70	8.0-9.0	8.5
F-20	A. naevia	Hemolymph	6-2-70	8.0	8.0
F-21	A. naevia	Hemolymph	6-2-70	7.0-8.0	7.6
F-22	L. reclusa	Venom	6-2-70	7.0-8.0	7.6
F-23	L. reclusa	Venom	6-2-70	7.0-8.0	7.6
F-24	L. reclusa	Venom	6-2-70	7.0-8.0	7.6
F-25	L. reclusa	Venom	6-2-70	7.0-8.0	7.6
F-25	L. reclusa	Hemolymph	6-2-70	7.0	6.8
M-26	L. reclusa	Venom	6-2-70	7.0-8.0	7.6
M-27	L. reclusa	Hemolymph	6-2-70	7.0-8.0	8.0 dropped to 6.8
M-28	L. reclusa	Gut	6-2-70	6.0	---
M-29	L. reclusa	Gut	6-2-70	6.0	---
M-30	L. reclusa	Gut	6-2-70	6.0	---

Hemolysis

Washed human red blood cells were prepared as previously described. The tube of washed red blood cells with 20 µl of venom and the control tubes were held overnight at room temperature. The following day the tube containing the venom showed no hemolysis while the controls also held at 85 F ± 5 showed total hemolysis after 24 hr.

To determine if a lower level of red cell lysis was present, fresh human blood was observed microscopically. A drop of fresh human blood was placed on a slide. A cover slip was placed over the blood drop. Two µl of venom were placed just under the edge of the cover slip with

a 10 μl syringe. An area radiating for 9 mm from the venom placement was immediately observed with the naked eye to lose its red color. Upon microscopic examination, red blood cell "ghosts" could be seen at the interface of the venom and blood. Identical results were obtained when this technique was used on whole canine blood. No morphological changes were noted on a control blood slide for a 2 hr observation period.

Microscopic observations show D. hentzi venom capable of human and canine red blood cell lysis. Hemolysis extended to few cells under the cover slip, thus the venom's efficacy as a hemolytic agent is probably very low. Since hemolysis was not seen in washed red blood cells, the hemolysis observed on the blood slides was probably due to osmotic activity.

Venom Gland Location

Preserved specimens of D. hentzi were dissected to locate the venom glands. A tarantula venom gland is pictured in Figure 16. The venom gland itself was located entirely in the chelicera (Figure 17). The material connected to the venom gland (Figure 17) and in the bottom of the chelicerae corresponds to branches of the hemolymph system pictured in Comstock (1948).

Preserved specimens of Loxosceles reclusa, Lycosa rabida, and Agelenopsis naevia were dissected in order to locate their venom glands to compare with D. hentzi. Venom glands for these spp. in contrast to the D. hentzi venom glands run from the chelicerae to approximately 3/4 of the way back into the cephalothorax. The brown spider had the largest venom glands in relation to its size, and the tarantula the smallest venom gland in relation to its size of the 4 spp. examined.

Tarantula Parasitism

Two cases of tarantula parasitism were observed. During April collecting 2 tarantulas were found shrivelled at the bottom of their burrows. Upon closer examination many small empty puparia about 2 to 3 mm long were found inside the tarantula carcasses. It was estimated that 100 to 300 puparia filled each carcass in the area of the book lungs.

As nearly as could be determined the tarantula parasite was of the Diptera family Cyrtidae sometimes called Acroceridae or Onodidae. According to Cole (1969) all known larvae of this family are internal parasites of spiders. However, no reference was found in the literature to Cyrtidae parasitism on tarantulas. Larvae of this family are reported by Cole to breathe through 2 posterior spiracles which were present on puparia collected in Oklahoma.

Cole described the life history of Cyrtidae. Usually eggs were laid in masses of 800 to 900 on the tips of vegetation. The larvae hatched when the foliage was moist and waited for a spider to come near. When the larvae attached to the body of a spider they gripped by the head and stuck out at a right angle from the body. Although the attacked spider often killed some of the larvae, most of the larvae were able to penetrate to the interior of the spider and devoured it slowly.

SUMMARY AND CONCLUSIONS

Areas where male tarantulas were seen roving provided an excellent place to start a tarantula hunt. Tarantula burrows were most often found in a hilly pasture near these roving areas. Approximately half way down a hill on the edges of small eroded bluffs or lightly vegetated areas were good places to find tarantula burrows. Immature tarantulas and mature female tarantulas were found in burrows with entrances 1.94 to 5.08 cm in diam. These burrows went straight down for 10 to 25.4 cm then turned at approximately a 45^{o} angle and continued for 20 to 91.5 cm more. All mature males were collected while they were migrating.

The smallest tarantula collected weighed .12 g while the largest tarantula collected weighed 17.54 g. The largest male collected weighed 10.2 g. However, the average weight of males collected was 5.54 g.

Male tarantulas were more fragile than females. Only 13% of the males collected in 1969 survived to June 1, 1970, while 33% of the females survived this long. Tarantulas with missing body parts were observed to replace those parts when they molted. Female tarantulas exhibited a behavioral characteristic of wrapping their insect debris in webbing and burying it under the sand while males did not. About one half the immatures wrapped their debris in webbing.

Males were observed to molt to the adult stage in the laboratory from June 1 to June 12. Adult males were recognized by their modified

palpi used for mating. The tarantula molt was a critical stage in the tarantula's life which often terminated in the death of the tarantula.

To obtain pure venom from tarantulas, small clear plastic tubes were placed over the fangs and the tarantula was given an electrical stimulus from a sine wave generator. The most venom collected at 1 time from a male was 11 μl while 1 female gave 34 μl of venom. With proper management female tarantulas could be "milked" once a week for 4 to 5 straight weeks and still maintain proper venom yield.

Two male migrations were observed in Oklahoma. These were May 24 to July 27 and Sept. 12 to 30. Females laid their eggs in the laboratory from June 19 to July 7.

Three rabbits injected subcutaneously in the hip with 10, 15, and 20 μl of venom showed no visible ill effects. However, mice injected at 1, 2, 3, 4, 5, 6, 7, and 9 μl of venom showed reactions to tarantula venom. Twenty mice were injected per treatment. At 6 hr, mice inoculated with 1, 2, 3, 4, 5, 6, 7, and 9 μl had 0%, 0%, 55%, 65%, 75%, 95%, 100%, and 100% mortality, respectively. The LD_{50} was about 0.28 μl venom/g body weight.

Tissues of the injected mice were examined for pathologic effects of the venom. Tissue slides of the skin, skeletal muscle, kidneys, adrenal glands, lungs, heart, intestine, liver and brain were examined microscopically. Acute lesions were observed within the first few hr after injection. These lesions were found in the skeletal muscle near the injection site and in the cardiac muscle. The lesions in the skeletal muscle were swelling of the muscle fibers and loss of cross striations (called Zenker's necrosis), separation of muscle bundles, and subcutaneous edema. In the heart the affected muscle fibers were

found in small groups and appeared more acidophilic than the surrounding normal fibers. The nuclei were small and dark.

The second type of lesions were subacute lesions found in mice which were sacrificed 72 hr after injection. They were seen in the same sites as the acute lesions. The muscle fibers at the injection site showed advanced stages of necrosis. The muscle cells were broken up and disoriented. The nuclei were very pyknotic. Very basophilic granular material (probably calcium) was deposited in the degenerating muscle. Inflammatory cells had infiltrated the injection site and edema was pronounced. The changes in the heart, which probably caused death, were similar except there were no inflammatory cells or edema. The mineral deposition was more pronounced, however.

Mice were injected with tarantula venom intravenously in the right femoral vein, intramuscularly in the right hip, subcutaneously in the chest, subcutaneously in the hip, and intracutaneously in the hip. All mice died and an average time of death was calculated. The average time of death for mice injected intravenously, intramuscularly, subcutaneously in the chest, subcutaneously in the hip, and intracutaneously in the hip were 45 seconds, 29 min, 46 min, 66 min, and 161 min, respectively. All mice were injected with 9 μl venom/10 g body weight except the mice injected intravenously which were given only 1.5 μl venom/10 g body weight. These results show that the faster the venom can get through the veins to the heart the faster the mouse will die. Thus, death was probably due to necrosis in the heart.

An LD_{50} was run on adult male American cockroaches injected with tarantula venom. At 6 hr the LD_{50} was 0.31 μl venom/roach.

No red blood cell lysis was seen when venom was placed in a tube of washed human red blood cells. However, when venom was placed on human and canine whole blood slides, a very low level of red blood cell lysis was observed.

Tarantula venom pH was found to be between 5.3 and 5.9 on a pH paper system. The pH of immature, mature male, and mature female tarantula venom was identical. Latrodectus mactans, Loxosceles reclusa, Agelenopsis naevia and Lycosa rabida venom pH's were 6.8, 7.6, 6.3, and 5.9, respectively. Venom pH for these 4 spp. was, also, identical from individual to individual for a sp. Possibly venom pH could be used to distinguish between live morphologically similar spp.

Little difference between female and male venom was observed in the visible stained protein components run through electrophoresis gels. The protein components for male and female tarantulas showed similar areas of toxicity to American cockroaches. Roach toxicity was observed in the top half of the venom electrophoresis gels with the peak toxicity observed in the area of the second visible band. Some of the gel slices in the top half of the gel showed temporary roach paralysis while others caused complete roach paralysis at 48 hr.

Similar results were obtained in later roach injections when a stacking gel was added. However, the stacking gel showed some roach toxicity. In June beetle injections toxic activity was exhibited by the venom components taken from gel slices B and D with these 2 slices and whole venom causing rapid internal cell breakdown in the June beetle.

When the electrophoresis poles were reversed toxic activity to roaches was found in the top half of the gel. Again, the stacking gel

was found to be toxic. The greatest toxicity was observed in the top .64 cm of the gel with descending toxicity as the slices moved down the gel.

Venom glands of the tarantula were found located entirely within the chelicerae. In contrast 3 other spider spp. examined had their venom glands extending as far as 2/3 of the way back into the cephalothorax. In all cases of the 4 spp. examined the venom glands were located dorsally. The brown spider had the largest venom glands relative to its size while the tarantula had the smallest.

Two cases of tarantula parasitism were observed. During April collecting 2 shrivelled tarantula carcasses were found at the bottom of burrows. About 100 to 300 empty puparia approximately 2 to 3 mm long were found inside the tarantula carcasses in the area of the book lungs. As nearly as could be determined, the tarantula parasites were of the Diptera, family Cyrtidae sometimes called Acroceridae or Onodidae.

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APPENDIX

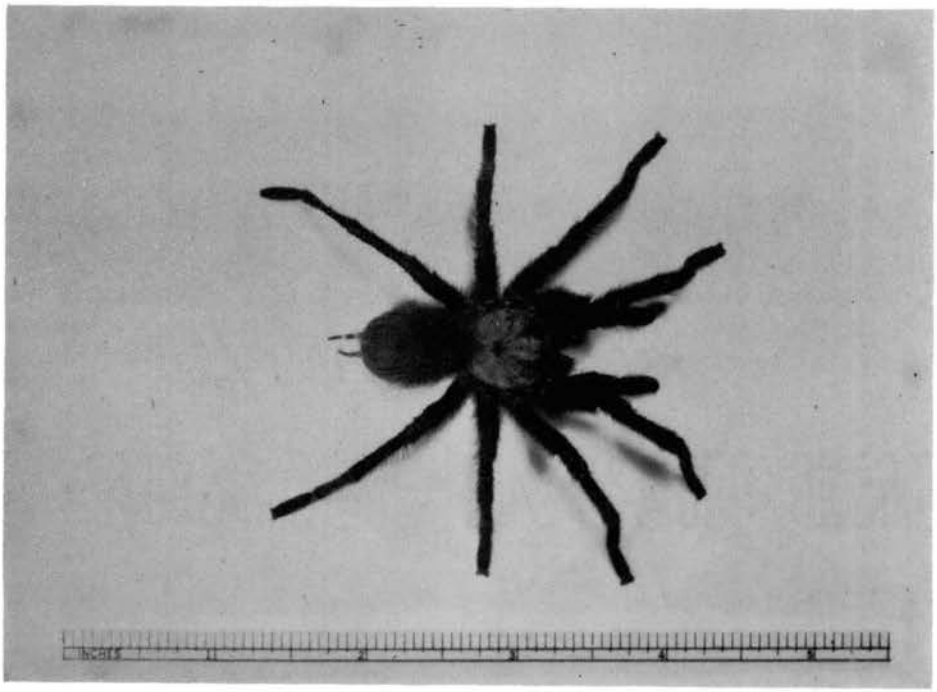

Figure 1. Male D. hentzi 2/3 natural size.

Figure 2. Female D. hentzi 2/3 natural size.

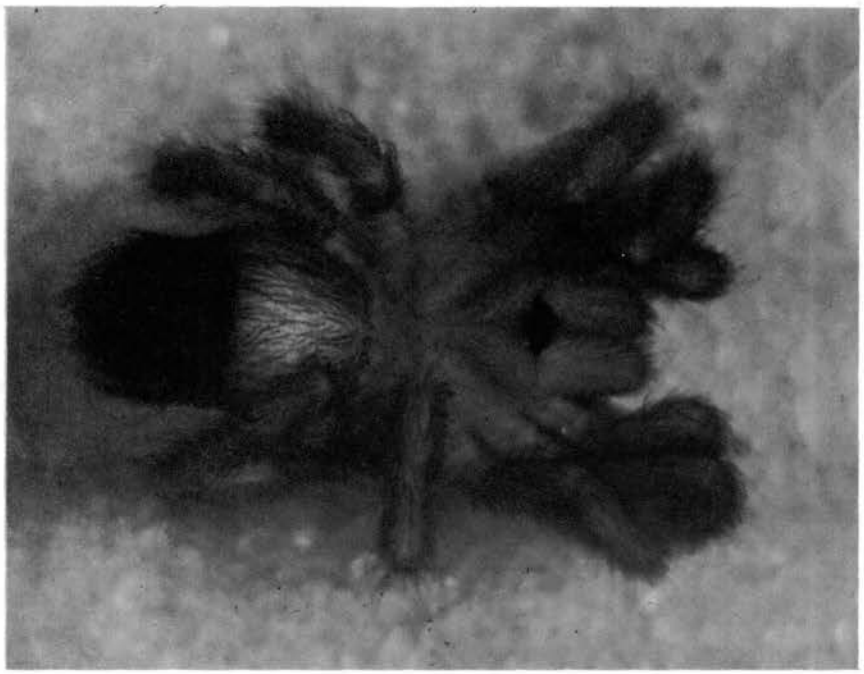

Figure 3. Newly hatched D. hentzi 30X natural size showing juvenile abdominal spot.

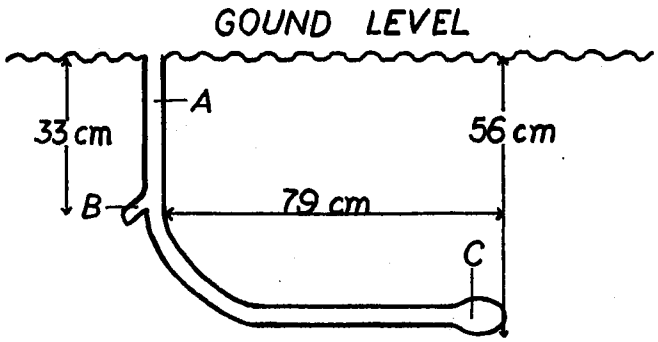

Figure 4. Diagram of a D. hentzi burrow.
A. Burrow
B. Molting chamber.
C. Egg laying chamber.

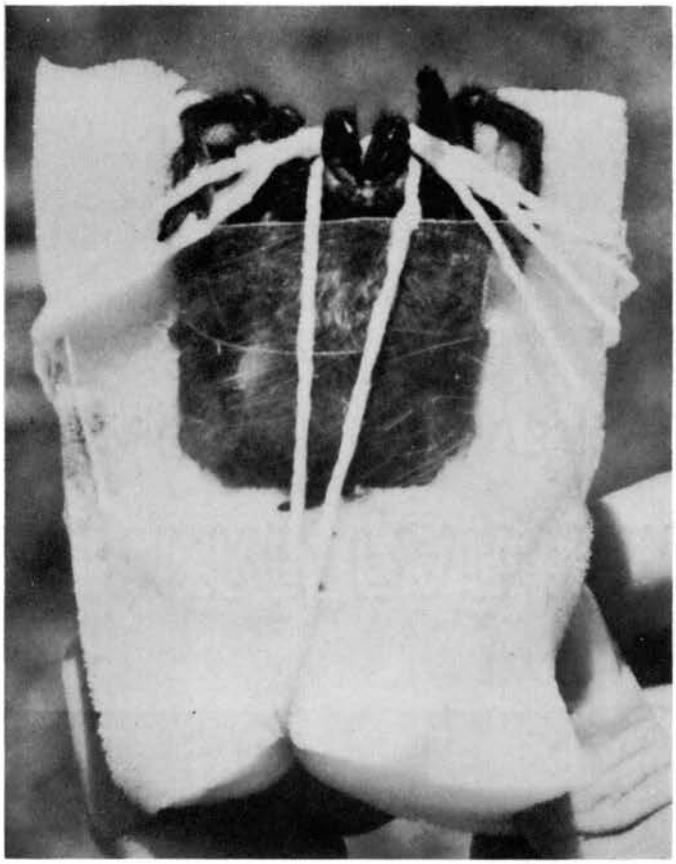

Figure 5. Method of D. hentzi restraint during "milking."

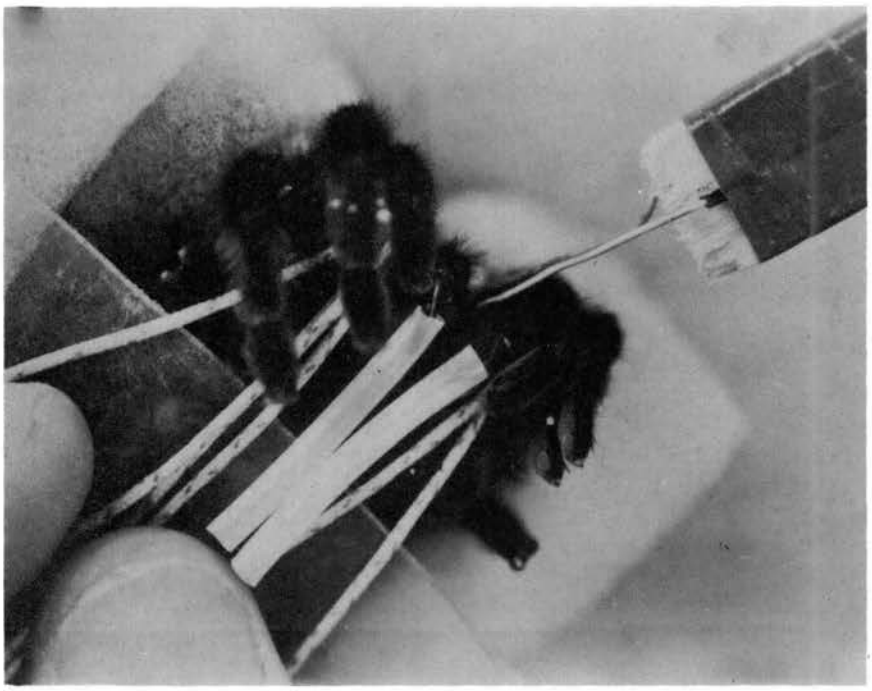

Figure 6. Collection of D. hentzi venom.

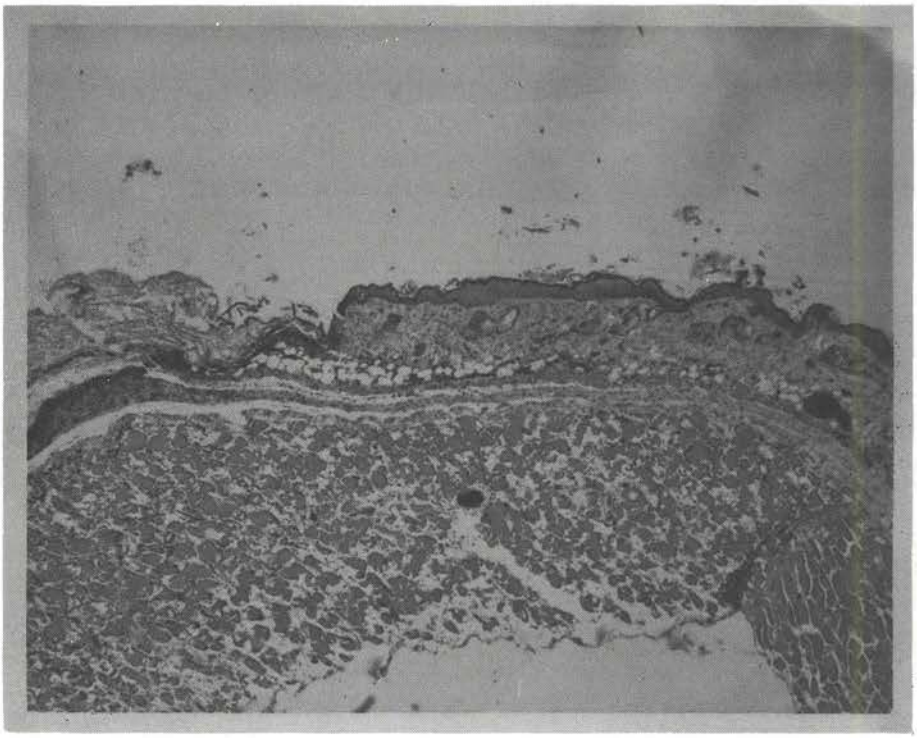

Figure 7. Tissue section of cuticle and skeletal muscle of a white mouse 66 hr after D. hentzi venom injection, showing necrotic areas to the left and normal tissues to the right (25X).

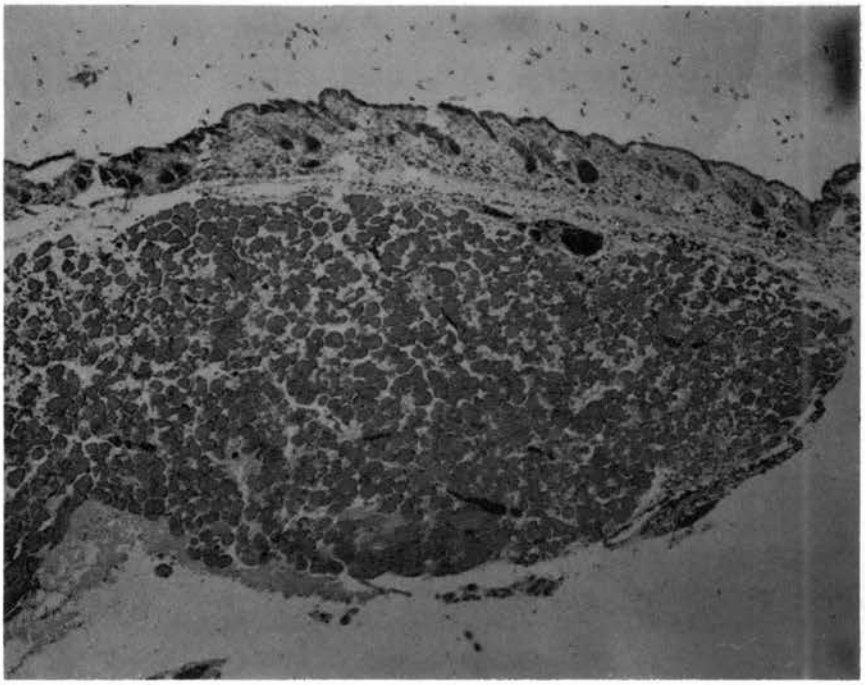

Figure 8. Tissue section of cuticle and skeletal muscle of white mouse 2 hr after D. hentzi venom injection, showing acute lesion in the skeletal muscle (25X).

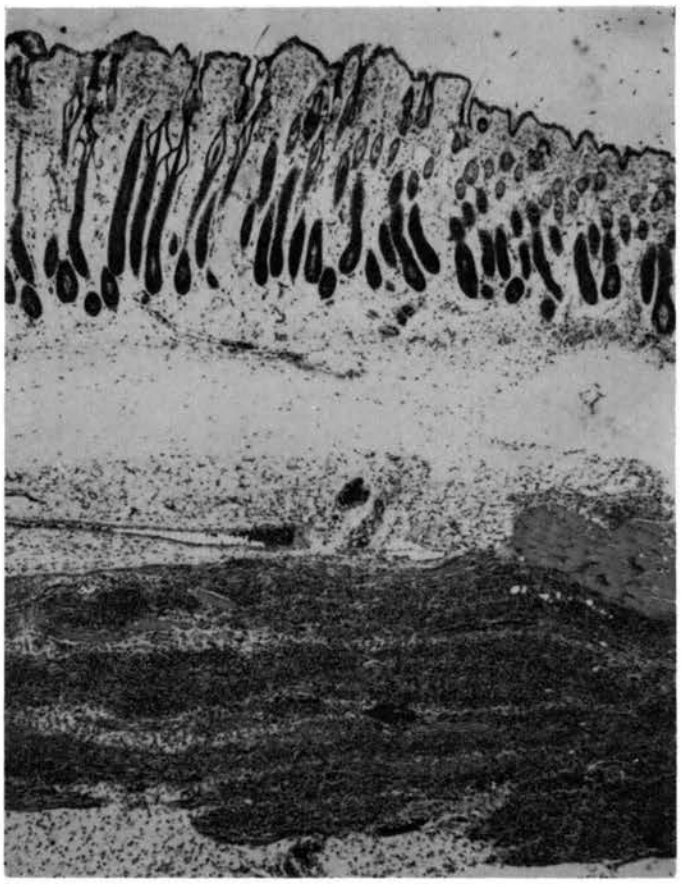

Figure 9. Tissue section of cuticle and skeletal muscle of a white mouse 72 hr after D. hentzi venom injection, showing subacute lesion (40X).

Figure 10. Tissue section of the heart of a white mouse 72 hr after D. hentzi injection, showing necrosis (25X).

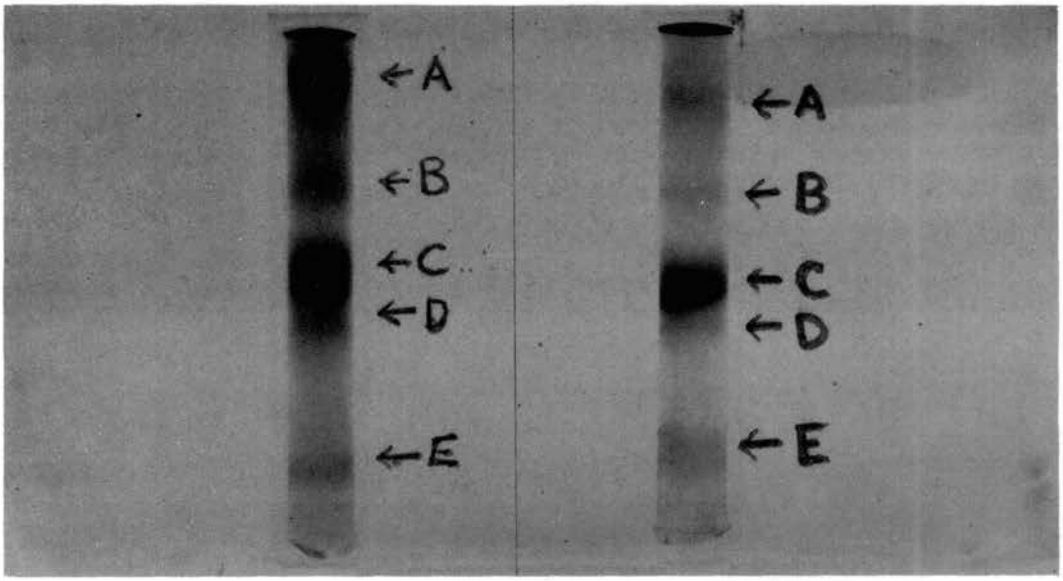

Figure 11. Electrophoretic pattern of venom of D. hentzi female (left) and male (right).

Figure 12. Electrophoretic pattern of D. hentzi venom with A, B, C, D, E, F, and S showing where the gel was sliced.

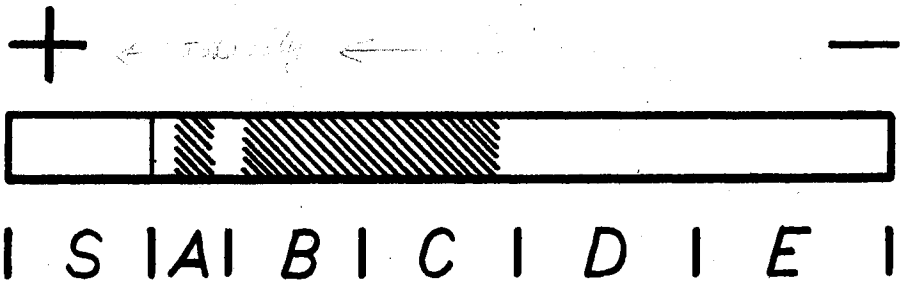

Figure 13. Electrophoretic pattern of D. hentzi venom with the positive and negative poles reversed, showing where the gel was sliced.

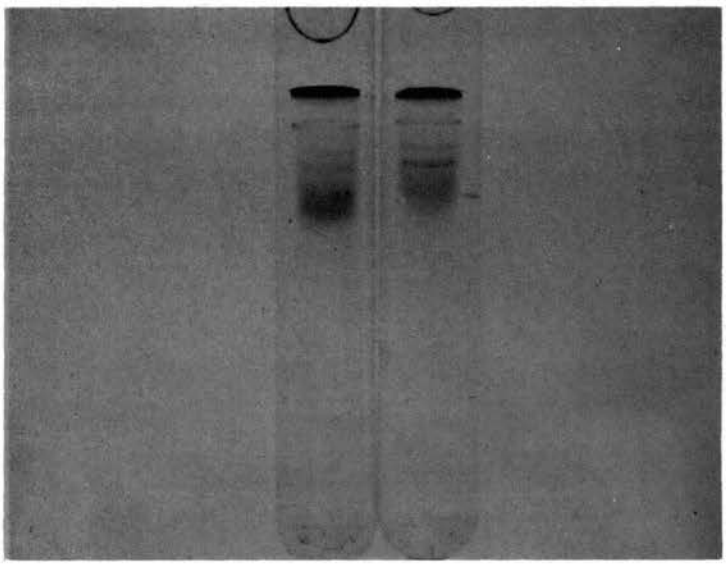

Figure 14. Electrophoretic pattern of
D. hentzi hemolymph.

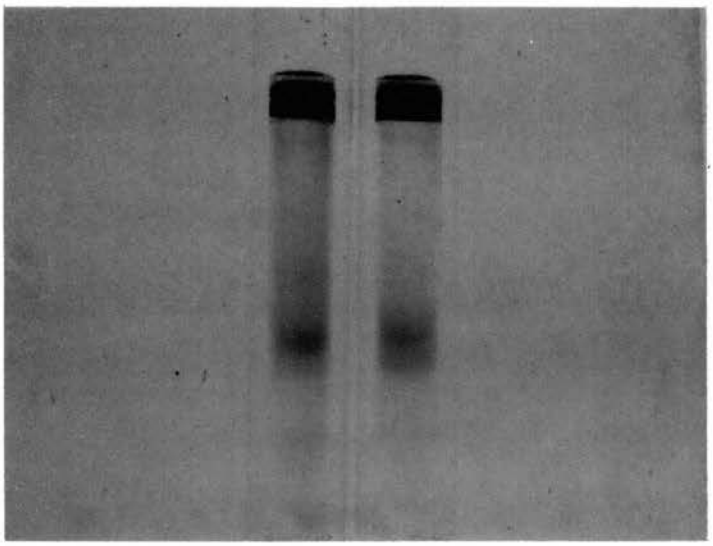

Figure 15. Electrophoretic pattern of
D. hentzi gut content.

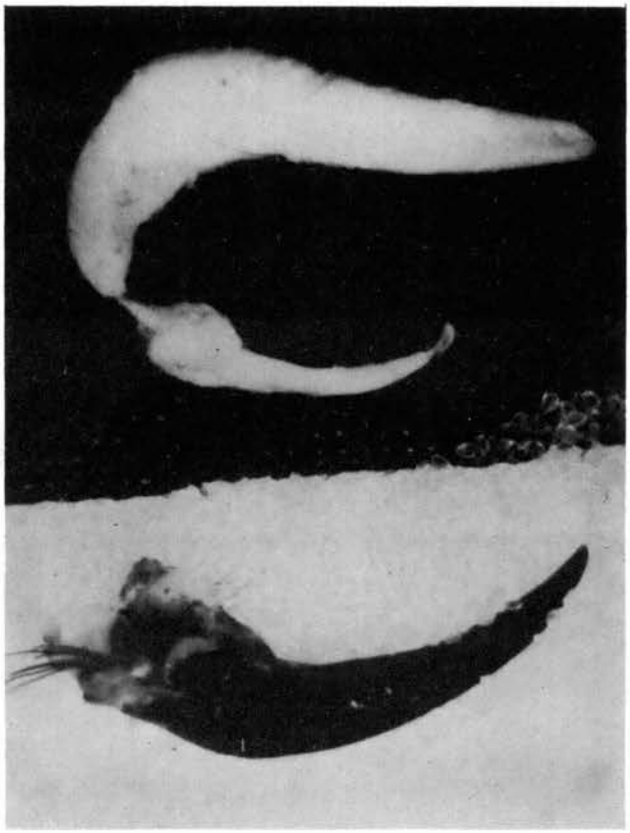

Figure 16. D. hentzi poison apparatus (30X). Upper- Bilobed poison gland removed from chelicerae. Lower- Distal portion of chelicera.

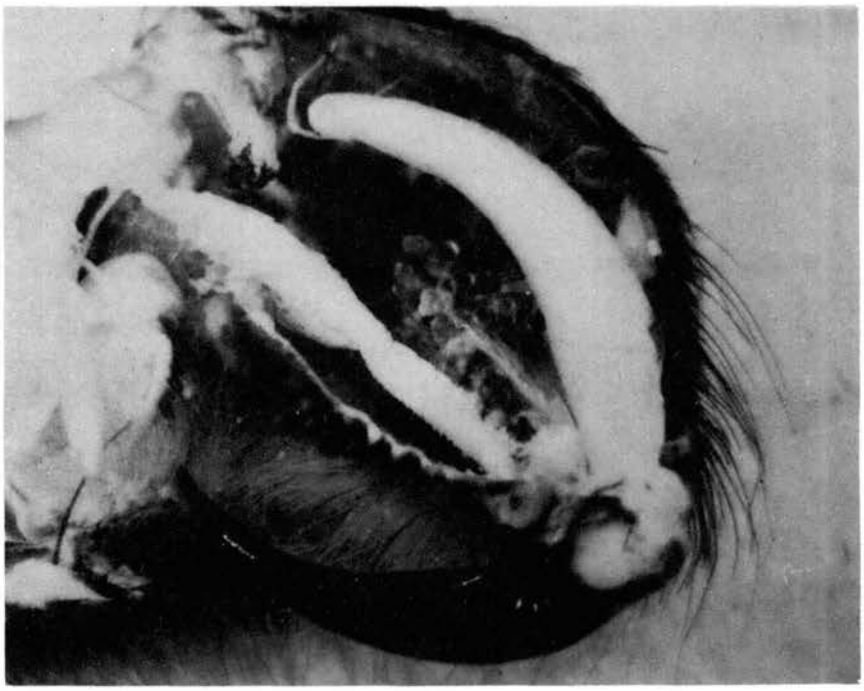

Figure 17. D. hentzi with mesial surface of chelicera removed to show location of the poison gland (30X).

APPENDIX B

COLLECTION SITE CODE

A. South Central Kansas.

B. Two miles west of Highways 15 and 281 junction (Oklahoma).

C. Cushing, Oklahoma.

D. Junction of Highways 18 and 64 (Oklahoma).

E. One mile east of Highways 51 and 77 junction (Oklahoma).

F. Highways I-35 and 51 junction (Oklahoma).

G. One mile west of Highway 86 on Highway 51 (Oklahoma).

H. Two miles north on Highway 51C off Highway 51 (Oklahoma).

I. One mile north on Highway 86 off Highway 51 (Oklahoma).

J. Yale, Oklahoma (in a basement).

K. Will Rogers Scout Reservation, 5 miles west of Cleveland, Oklahoma.

L. Southeastern Oklahoma on the highway.

M. Nine miles east of Stillwater, Oklahoma, on Highway 51.

N. Two miles west of Highways I-35 and 51 junction (Nida farm).

O. Four miles north of Stillwater, Oklahoma, on Highway 177.

P. Three miles west of Pawnee, Oklahoma (Sayer farm).

Q. Pawnee, Oklahoma.

R. KSPI radio tower two miles south of Stillwater, Oklahoma.

S. Stillwater, Oklahoma.

Table 13. Biological data for male *D. hentzi*.

Spider ident.	Date collected	Times milked	Avg. µl. venom/ milking	Date molted	Coll. wt. in g	Date of death
S-1	5-24-69	4	2	-	-	3-24-70
S-2	6-18-69	5	2	-	-	9-1-69
S-3	6-19-69	2	2	-	-	9-1-69
S-4	6-23-69	4	2	-	-	9-20-69
S-5	6-25-69	1	2	-	-	7-30-69
K-6	7-2-69	2	2	-	-	9-20-69
P-7	7-11-69	6	2	6-1-70	6.73	6-23-70
S-8	7-15-69	4	2	2-17-70	-	3-24-70
S-9	7-20-69	2	2	-	-	10-27-69
S-10	7-27-69	2	2	-	-	9-30-69
L-11	9-12-69	-	-	-	-	3-18-70
L-12	9-12-69	-	-	-	-	3-23-70
C-13	9-14-69	-	-	-	-	2-23-70
S-14	9-15-69	1	7	-	-	3-10-70
S-15	9-15-69	1	1	-	-	3-24-70
S-16	9-15-69	-	-	-	-	4-14-70
Q-17	9-19-69	1	3	-	-	3-24-70
S-18	9-20-69	1	1	-	4.03	-
S-19	9-20-69	-	-	-	-	3-20-70
S-20	9-20-69	4	3	-	7.19	-
S-21	9-23-69	1	1	-	-	11-18-69
S-22	9-25-69	-	-	-	-	11-27-69
S-23	9-29-69	-	-	-	-	2-20-70

Table 13 (Continued)

Spider ident.	Date collected	Times milked	Avg. µl venom/ milking	Date molted	Coll. wt. in g	Date of death
S-24	9-30-69	-	-	-	-	2-15-70
S-25	9-30-69	-	-	-	-	2-20-70
P-26	4-20-70	1	9	6-1-70	10.20	7-16-70
P-27	4-20-70	1	5	6-1-70	6.87	6-3-70
J-28	4-24-70	3	4	6-1-70	5.12	7-24-70
J-29	4-27-70	1	0	6-1-70	5.89	6-1-70
G-30	4-27-70	-	-	6-1-70	4.92	-
G-31	4-27-70	-	-	6-12-70	3.86	-
G-32	4-27-70	2	5	6-12-70	5.71	7-24-70
G-33	6-10-70	1	7	-	5.17	-
S-34	6-10-70	1	3	-	6.76	7-24-70
E-35	6-11-70	2	4	-	4.54	-
N-36	6-13-70	2	8	-	5.77	7-8-70
G-37	6-18-70	-	-	-	4.31	6-23-70
G-38	6-18-70	1	7	-	5.17	6-21-70
S-39	6-19-70	2	4	-	8.33	7-30-70
S-40	6-19-70	2	0	-	5.08	7-20-70
N-41	6-19-70	2	5	-	4.00	7-8-70
N-42	6-19-70	1	3	-	5.84	7-24-70
G-43	6-20-70	1	6	-	-	7-24-70
G-44	6-20-70	2	5	-	-	7-17-70
S-45	6-22-70	-	-	-	-	7-2-70
S-46	6-22-70	2	2	-	4.84	-

Table 13 (Continued)

Spider ident.	Date collected	Times milked	Avg. µl venom/ milking	Date molted	Coll. wt. in g	Date of death
S-47	6-22-70	3	1	-	3.87	-
F-48	6-23-70	1	4	-	5.94	-
S-49	6-24-70	3	4	-	6.26	7-30-70
S-50	6-24-70	1	6	-	8.38	7-21-70
A-51	6-24-70	1	6	-	5.64	7-24-70
A-52	6-24-70	1	11	-	9.30	7-30-70
M-53	6-25-70	-	-	-	-	7-2-70
M-54	6-25-70	-	-	-	-	7-2-70

Table 14. Biological data for immature *D. hentzi*[a].

Spider ident.	Date collected	Times milked	Avg. µl venom/ milking	Date molted	Coll. wt. in g	Date of death
G-1	7-12-69	-	-	-	-	7-12-69
S-2	3-21-70	-	-	-	-	3-21-70
G-3	4-24-70	-	-	-	-	4-24-70
G-4	4-24-70	-	-	-	2.10	-
G-5	4-24-70	-	-	6-30-70	2.64	-
G-6	4-27-70	-	-	7-29-70	2.51	-
G-7	4-27-70	-	-	6-20-70	2.68	-
G-8	4-27-70	-	-	-	3.46	-
G-9	4-27-70	-	-	-	3.86	-
B-10	5-14-70	-	-	-	2.69	-
B-11	5-14-70	-	-	-	1.66	-
B-12	5-14-70	-	-	-	2.41	-
S-13	5-20-70	-	-	-	2.07	-
J-14	6-1-70	-	-	7-8-70	2.84	-
G-15	6-11-70	-	-	-	1.69	-
N-16	6-13-70	3	1	-	3.73	-
N-17	6-19-70	-	-	-	2.69	-
N-18	6-19-70	-	-	-	2.11	-
N-19	6-19-70	-	-	-	2.11	-
N-20	6-19-70	-	-	-	3.13	-
N-21	6-19-70	-	-	6-23-70	3.32	-
N-22	6-20-70	-	-	-	2.47	-
N-23	6-22-70	-	-	-	2.91	-

Table 14 (Continued)

Spider ident.	Date collected	Times milked	Avg. µl venom/ milking	Date molted	Coll. wt. in g	Date of death
N-24	6-22-70	-	-	-	2.80	-
N-25	6-22-70	-	-	-	3.83	-
N-26	6-22-70	-	-	-	3.73	-
I-27	7-6-70	2	5	7-7-70	3.35	-

[a]Any spider weighing less than 4.00 g was considered immature.

Table 15. Biological data for female D. hentzi.

Spider ident.	Date collected	Times milked	Avg. µl venom/ milking	Date molted	Coll. wt. in g	Date of death
S-1	8-14-68	4	2	-	-	8-12-69
S-2	8-16-68	2	4	8-18-69	-	1-5-70
S-3	5-11-69	2	3	-	-	7-7-69
S-4	5-15-69	3	4	7-27-69	-	9-11-69
P-5	7-11-69	4	2	-	-	5-3-70
P-6	7-11-69	2	3	9-10-69	-	3-10-70
P-7	7-11-69	2	1	-	-	8-18-69
P-8	7-11-69	2	1	9-1-69	-	3-12-70
P-9	7-11-69	1	3	9-1-69	-	10-3-69
T-10	7-13-69	3	6	-	-	2-14-70
S-11	8-5-69	3	6	4-17-70	-	5-3-70
P-12	8-11-69	1	0	9-1-69	-	12-3-69
P-12	8-11-69	1	7	-	-	3-12-70
P-13	8-11-69	3	4	3-10-70	-	3-10-70
P-14	8-11-69	1	7	-	-	3-12-70
P-15	8-11-69	4	4	2-15-70	-	4-6-70
P-16	8-11-69	1	7	-	-	3-5-70
P-17	8-11-69	-	-	-	-	9-17-69
P-18	8-18-69	-	-	-	-	9-30-69
0-19	8-18-69	-	-	-	-	12-6-69
T-20	8-21-69	-	-	-	-	9-1-69
F-21	4-17-70	6	4	7-22-70	4.81	-
F-22	4-17-70	2	6	7-16-70	4.94	-

Table 15 (Continued)

Spider ident.	Date collected	Times milked	Avg. µl venom/ milking	Date molted	Coll. wt. in g	Date of death
P-23	4-20-70	3	4	-	4.95	-
G-24	4-27-70	3	6	-	4.34	7-16-70
G-25	4-27-70	2	6	7-30-70	4.87	-
G-26	4-27-70	1	2	7-3-70	4.54	-
G-27	4-27-70	2	5	-	4.82	-
G-28	4-27-70	1	2	-	4.96	-
B-29	5-14-70	6	8	-	4.58	-
E-30	6-11-70	-	-	7-3-70	4.23	-
N-31	6-13-70	3	5	-	4.71	-
N-32	6-13-70	5	3	-	4.74	-
N-33	6-16-70	3	4	7-31-70	4.84	-
N-34	6-16-70	-	-	6-24-70	4.08	-
N-35	6-16-70	3	4	-	4.75	-
N-36	6-19-70	-	-	7-3-70	4.00	-
N-37	6-19-70	-	-	-	4.37	-
N-38	6-22-70	4	7	-	4.60	-
N-39	6-22-70	3	12	-	4.67	-
N-40	6-22-70	2	10	-	4.26	-
I-41	7-6-70	2	7	-	4.58	-
I-42	7-7-70	1	10	7-13-70	4.81	-
I-43	7-7-70	3	4	-	4.42	-
I-44	7-7-70	2	5	-	4.77	-
G-45	4-27-70	2	5	7-21-70	5.16	7-21-70

Table 15 (Continued)

Spider ident.	Date collected	Times milked	Avg. µl venom/ milking	Date molted	Coll. wt. in g	Date of death
G-46	4-27-70	3	7	7-16-70	5.32	-
G-47	4-27-70	2	8	6-1-70	5.76	-
G-48	6-10-70	3	1	7-3-70	5.36	-
E-49	6-11-70	-	-	6-20-70	5.21	-
N-50	6-13-70	2	12	-	5.05	-
N-51	6-13-70	2	7	7-3-70	5.43	-
N-52[a]	6-13-70	3	4	-	5.55	-
N-53	6-16-70	6	5	-	5.90	-
N-54	6-19-70	3	4	-	5.16	-
I-55	7-7-70	2	10	-	5.03	-
I-56	7-7-70	2	8	-	5.99	-
I-57	7-7-70	2	8	-	5.59	-
H-58	7-22-70	2	6	-	5.99	-
H-59	7-22-70	1	11	-	5.45	-
G-60	7-22-70	1	5	-	5.45	-
G-61	7-22-70	1	20	-	5.93	-
P-62	7-11-69	7	6	-	6.93	-
G-63	4-27-70	5	8	-	6.03	-
G-64	6-10-70	4	10	-	6.32	-
N-65	6-13-70	2	5	-	6.93	-
N-66[a]	6-13-70	2	3	-	6.09	-
E-67	6-18-70	3	9	-	6.83	-
S-68	6-18-70	1	7	-	6.29	-

Table 15 (Continued)

Spider ident.	Date collected	Times milked	Avg. µl venom/ milking	Date molted	Coll. wt. in g	Date of death
N-69[a]	6-19-70	2	7	-	6.51	-
N-70	6-22-70	4	11	-	6.90	-
D-71	6-30-70	1	10	-	6.15	-
I-72	7-6-70	2	10	-	6.42	-
I-73	7-7-70	2	7	-	6.67	-
I-74	7-7-70	2	8	-	6.09	-
I-75	7-7-70	2	17	-	6.01	6-23-70
P-76	7-11-70	5	5	-	6.73	-
G-77	7-22-70	1	6	-	6.33	-
P-78	4-20-70	1	2	-	7.18	-
G-79	4-24-70	6	14	-	7.06	-
G-80	4-27-70	3	8	-	7.10	-
G-81	4-27-70	4	16	-	7.44	-
G-82	4-27-70	7	10	-	7.86	-
B-83	5-14-70	7	3	-	7.90	-
G-84	6-10-70	4	5	-	7.90	-
G-85	6-10-70	5	9	-	7.89	-
E-86	6-11-70	3	12	-	7.81	7-31-70
N-87	6-13-70	-	-	-	7.91	-
G-88	6-15-70	1	5	-	7.00	-
N-89	6-20-70	3	4	-	7.90	-
F-90[a]	6-22-70	2	14	-	7.37	-
D-91	7-1-70	-	-	-	7.84	-

Table 15 (Continued)

Spider ident.	Date collected	Times milked	Avg. µl venom/ milking	Date molted	Coll. wt. in g	Date of death
I-92	7-6-70	2	12	-	7.00	-
I-93	7-6-70	2	10	-	7.18	-
I-94[a]	8-3-70	-	-	-	7.82	-
F-95	4-25-70	2	6	-	8.05	-
G-96	4-27-70	4	7	-	8.05	-
N-97	6-13-70	3	7	-	8.60	-
N-98[a]	6-13-70	3	9	-	8.60	-
N-99	6-16-70	5	8	-	8.94	-
N-100	6-16-70	3	7	-	8.33	-
N-101	6-16-70	2	12	-	8.32	6-22-70
N-102	6-16-70	5	6	-	8.89	-
N-103	6-19-70	-	-	-	8.05	-
N-104[a]	6-19-70	3	10	-	8.46	-
N-105	6-20-70	3	15	-	8.78	-
D-106	6-30-70	2	8	-	8.10	-
D-107	7-6-70	2	12	-	8.08	-
I-108[a]	8-3-70	-	-	-	8.65	-
F-109	8-21-70	8	4	-	9.34	-
I-110	6-10-70	5	15	-	9.23	-
N-111	6-13-70	6	7	-	9.81	-
N-112	6-13-70	3	10	-	9.49	-
N-113	6-13-70	4	10	-	9.92	-
N-114	6-16-70	4	7	-	9.31	-

Table 15 (Continued)

Spider ident.	Date collected	Times milked	Avg. µl venom/ milking	Date molted	Coll. wt. in g	Date of death
N-115	6-16-70	5	10	-	9.24	-
N-116	6-16-70	3	7	-	9.33	-
N-117	6-16-70	5	7	-	9.92	-
R-118	6-30-70	3	8	-	9.51	-
R-119	6-30-70	4	9	-	9.26	-
G-120	7-22-70	1	23	-	9.21	-
F-121	4-17-70	3	9	-	10.08	-
P-122	4-20-70	4	4	-	10.29	-
B-123	5-14-70	7	12	-	10.55	-
N-124	6-13-70	6	9	-	10.40	-
N-125[a]	6-13-70	4	7	-	10.19	-
N-126	6-13-70	5	8	-	10.15	-
N-127	6-16-70	1	3	-	10.95	7-6-70
N-128	6-16-70	5	12	-	10.10	-
N-129	6-16-70	5	7	-	10.07	-
N-130	6-19-70	4	6	-	10.95	-
N-131	6-19-70	4	8	-	10.32	-
N-132	6-19-70	5	18	-	10.83	-
N-133	6-20-70	3	7	-	10.00	7-31-70
N-134	6-30-70	3	9	-	10.00	-
E-135	7-16-70	11	8	8-6-70	10.63	-
E-136	6-11-70	4	20	-	11.77	-
N-137	6-13-70	4	5	-	11.79	7-30-70

Table 15 (Continued)

Spider ident.	Date collected	Times milked	Avg. µl venom/ milking	Date molted	Coll. wt. in g	Date of death
N-138	6-13-70	5	8	-	11.60	-
N-139	6-13-70	2	7	-	11.70	-
N-140	6-16-70	5	8	-	11.34	-
N-141	6-19-70	4	15	-	11.49	7-21-70
N-142	6-19-70	3	10	-	11.97	-
F-143	8-18-69	4	5	4-6-70	12.34	-
S-144	6-14-69	1	7	8-16-70	12.31	-
G-145	4-27-70	2	12	7-31-70	12.12	-
E-146[a]	6-11-70	4	15	-	12.55	-
N-147	6-13-70	4	8	-	12.73	-
A-148	6-15-70	5	12	-	12.47	-
N-149[a]	6-20-70	3	12	-	12.45	-
N-150	6-22-70	-	-	7-16-70	12.88	7-16-70
S-151	6-26-69	2	11	9-1-69	14.21	-
P-152	8-11-69	1	12	9-1-69 & 6-10-70	13.26	-
O-153[a]	8-18-69	1	7	-	14.04	-
S-154	4-27-70	5	14	-	17.54	-
S-155	6-18-70	6	7	-	13.69	-
N-156[a]	6-20-70	5	17	-	13.50	-
N-157[a]	6-20-70	5	25[b]	-	16.08	-
F-158	6-22-70	3	11	-	16.27	-

[a]Egg sac.

[b]Gave 34 microliters of venom at 1 "milking."

VITA 3

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