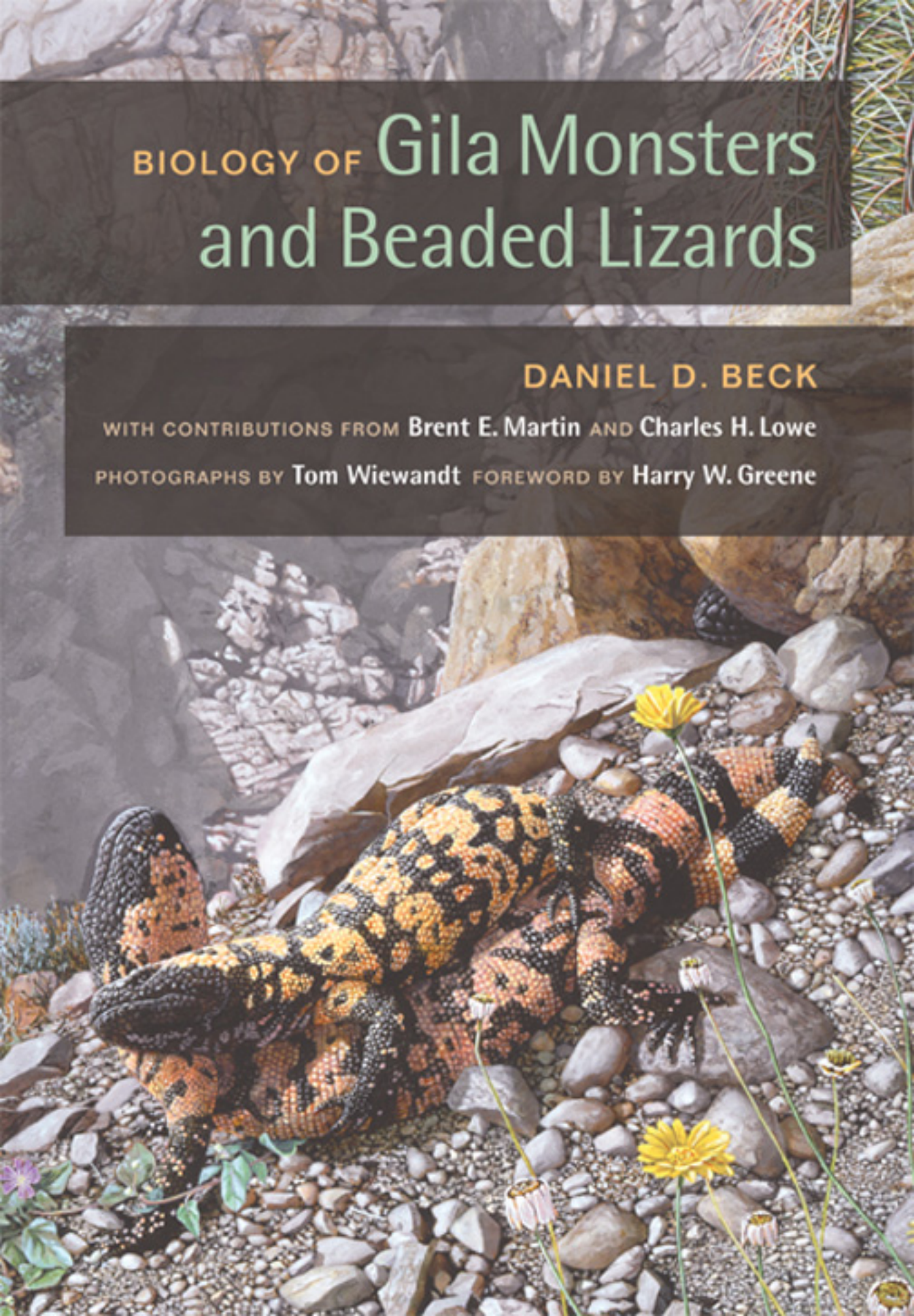
BIOLOGY OF Gila Monsters and Beaded Lizards

DANIEL D. BECK

WITH CONTRIBUTIONS FROM Brent E. Martin AND Charles H. Lowe

PHOTOGRAPHS BY Tom Wiewandt FOREWORD BY Harry W. Greene



CHAPTER 3

The Venom System and Envenomation

THE MOST WIDELY recognized feature of the Helodermatidae family is that its members are venomous. Misunderstanding and confusion about this trait have accompanied *Heloderma* since before the genus was described by Hernández in 1577. As discussed in chapter 1, the specific epithet, *suspectum,* was chosen for the Gila Monster because E. D. Cope *suspected* it was venomous (Cope 1869), but it was not until well into the twentieth century that scientists agreed that, indeed, this was true. Debate continues over whether the venom is used for prey acquisition or defense, and, more recently, promise has arisen over its potential applications in modern medicine and pharmacology.

In this chapter, I outline the structure and function of the venom delivery system of helodermatid lizards. I provide a historical overview, synthesize recent developments in venom biochemistry, and consider the function of the venom system in its ecological context. I conclude the chapter with an overview of envenomation history and the treatment of bites.

HISTORICAL OVERVIEW

Several authors in the latter half of the nineteenth century commented on the "vile nature" of *Heloderma* and recognized that its unique grooved dentition might be associated with a venomous bite (see Bogert and Martín del Campo 1956). But it was not until the 1880s that the anatomy of the venom gland was investigated (Fischer 1882), and experiments were conducted to show that its salivary secretions were, in fact, toxic to other animals (Mitchell and Reichert 1883). Instead of resolving uncertainty about the venomous nature of *Heloderma,* however, this work served to initiate a controversy that lasted for several decades. Subsequent experiments (Yarrow 1888) failed to show toxic effects of *Heloderma* venom, probably because inappropriate methods were used to collect the venom. Additional investigators examined the histology of the venom gland (Holm 1897) and showed, with controls, that carefully extracted venom injected into small vertebrates had lethal effects (Santesson 1897; Van Denburgh 1898; Van Denburgh and Wight 1900). Nevertheless, skepticism remained. Snow (1906), having suffered a bite without experiencing serious pain or swelling, again raised the question of whether *Heloderma* was venomous. In 1907, Goodfellow made the following statement: "... exhaustive studies were made by some of the attaches of the Smithsonian Institution, among whom was Dr. R. E. Shufeldt, concerning the nature of the animal, and conclusions reached which the writer had previously attained—that the reptile was non-venomous; and it may be accepted as conclusively demonstrated that the bite of the "monster" is innocuous per se. In 1913, an authoritative,

244-page book summarizing detailed studies of the venom of *Heloderma* was published by the Carnegie Institution of Washington (Loeb et al. 1913). This book contained detailed studies by 11 contributors on numerous aspects of the venom, including its biochemistry and effects on various physiological systems in a number of organisms. By the 1920s, this book, and additional studies on the venom gland, venom effects, and venom characteristics by Phisalix (1911, 1912, 1917, 1922), finally convinced the scientific community that helodermatid lizards were indeed venomous, and the debate was settled over the "suspected" venomous nature of *Heloderma*.

VENOM DELIVERY SYSTEM

Heloderma delivers venom through an efficient system consisting of paired venom glands that empty through ducts at the base of venom-conducting teeth. Venom is produced and housed in multilobed venom glands (fig. 9), which, unlike those of venomous snakes, are located in the lower jaw and drain through ducts associated with each of the lobes. In contrast, the venom glands of snakes are situated behind the eye, above the upper jaw, and drain through a single duct that leads to an opening at the base of the associated fang (Greene 1997). The venom glands of *Heloderma* are not surrounded by compressor musculature as in most venomous snakes. Instead, tension within the glands produced by jaw movements propels venom toward the venom-conducting teeth, and capillary action carries the venom from grooved teeth into the wound.

The structure of the venom gland was first described by Fischer (1882), but the most complete descriptions to date remain the work of Fox (1913) and Phisalix (1912, 1917). More recent reviews (e.g., Bogert and Martín del Campo 1956; Tinkham 1971b; Russell and Bogert 1981), as well as what I provide below, are largely summaries of this earlier work.

The venom glands of helodermatid lizards are visible externally as conspicuous swellings below the lower lips (fig. 9, plate 20). Each gland is surrounded by a fibrous capsule from which septa extend to divide the gland into three or four distinct lobes (fig. 9). Each lobe of the venom gland is a structurally independent organ that forms a sac with a swollen glandular region at its base and a narrowed excretory duct near the upper end, which empties at the base of the teeth in the lower jaw (fig. 10). Each lobe of the venom gland is subdivided (by septa) into several lobules, which are further subdivided into smaller lobules. These subdivisions continue to occur, resulting in tiny chambers, or alveoli, each separated from one another by a delicate septum. The cavities within the alveoli are continuous with the lobules, which are, in turn, continuous with one another, forming a network of intralobular tubules. It is within these structures, apparently, that venom is produced by columnar, granule-secreting cells that

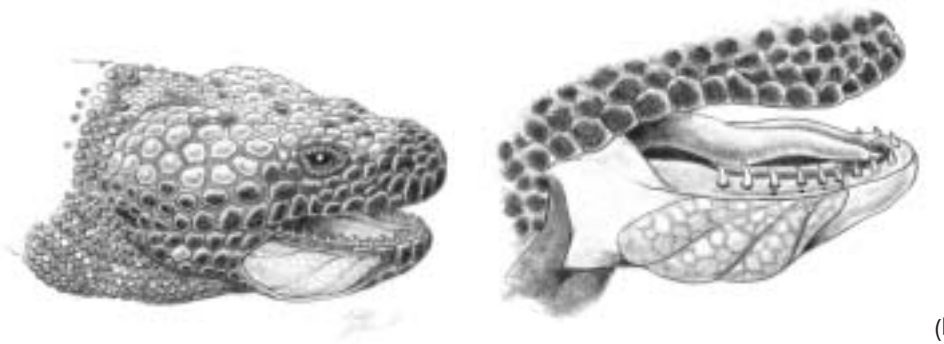


FIGURE 9. The venom glands of helodermatid lizards are located in the lower jaw. Each gland comprises three or four distinct lobes as shown in these 2 views of a Gila Monster's right lower mandible with the skin removed (drawings by Randy Babb).

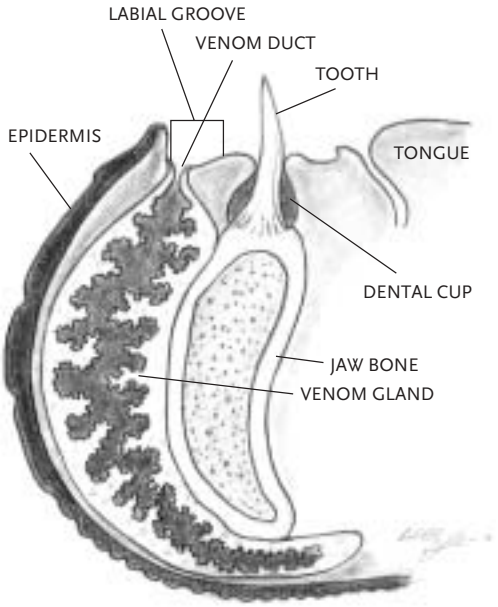


FIGURE 10. This cross section through the anterior portion of the right mandible shows relationships of the primary structures of the venom delivery system of helodermatid lizards. Each lobe of the venom gland forms a sac with a narrowed excretory duct near its upper end. The duct empties at the base of venom-conducting teeth in the lower jaw.

line the intralobular tubules. As these cells discharge their contents, the secreted granules flow from the tubules into a central collecting lumen, which connects to the excretory duct. The lumen, along with associated tubules and alveoli, apparently also serves as a storage reservoir for venom.

Helodermatids are not specialized for injecting large quantities of venom during brief contact, as are many venomous snakes, but the venom delivery system is structured to quickly and effectively transfer venom into a bite. During biting, tension produced in the gland by jaw movements propels venom through the venom ducts into a region between the fourth and seventh pair of dentaries (counting from the front), where the teeth show their greatest specialization for piercing and venom delivery. A series of small folds and grooves in the membranous tissue within this region serves as a temporary reservoir for the venom and may facilitate the flow of venom from duct to tooth. When the lizard bites, venom flows from these reservoirs through the grooved teeth into the wound.

Each specialized tooth has two grooves, with a shallower (sometimes absent) groove toward the rear (fig. 11) . Each groove is flanked by a cutting flange, which makes the tooth better adapted for piercing flesh than a merely conical tooth. Not all the teeth are similar in structure or size. The largest, most deeply grooved teeth are the dentaries (in the lower jaw), which can be up to 6 mm long in *H. horridum* and 5 mm long in *H. suspectum* (fig. 11). The maxillary

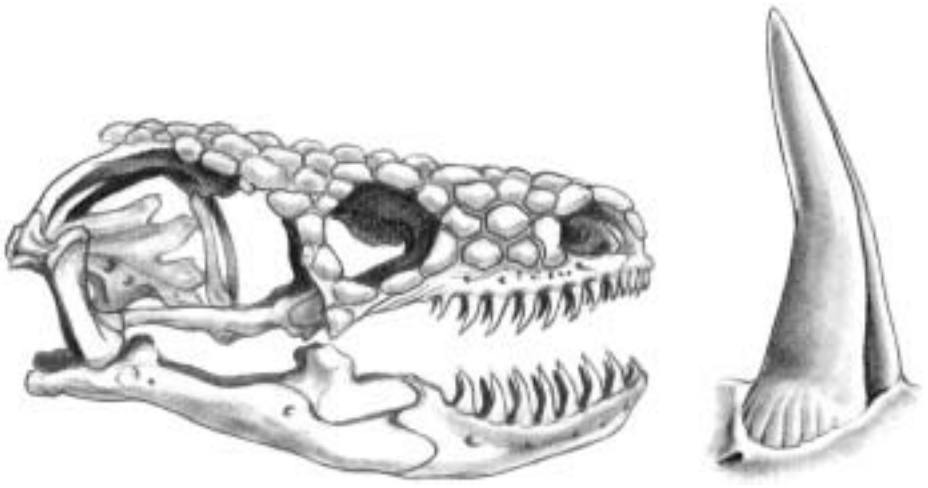


FIGURE 11. Skull of *Heloderma suspectum* showing arrangement of venom-conducting teeth (top). The largest, most deeply grooved teeth are the dentaries (in the lower jaw), which can be up to 5 mm long in *H. suspectum* (drawings by Randy Babb).

teeth (in the upper jaw) are shorter and less strongly grooved. Some teeth (especially the premaxillaries toward the front of the mouth) are hardly grooved at all, but they can still effectively deposit venom into an adversary (Tinkham 1956; Strimple et al. 1997).

Jaw movements other than those associated with biting may also produce sufficient tension within the glands to bathe all the teeth in venom. An agitated lizard will often display a defensive posture of opening its jaws wide (exposing the purplish-white interior of its mouth) then closing and reopening the jaws as a threat continues. This behavior may serve to bathe the maxillary teeth, dentaries, and premaxillaries in venom preparatory to envenomation.

The quantity of venom deposited into the victim may vary with many factors, including size of the lizard, degree of agitation, and length of time it remains attached. In captive Gila Monsters, quantities ranging from 0.5 to 2.0 ml in a single milking have been extracted by a variety of methods (Loeb et al. 1913; Arrington 1930; Brown and Lowe 1955; Alagón et al. 1982; fig. 12).

EFFECTS OF THE VENOM

The most complete investigation of the effects of *Heloderma* venom remains the work of Loeb et al. (1913) who tested hundreds of species, including invertebrates. Invertebrates are essentially immune to the effects of *Heloderma* venom. Effects on vertebrates are more severe and varied. Ectotherms appear markedly less susceptible to the effects of the venom than do endotherms (Cooke and Loeb 1913). Notably, Gila Monsters appear to be immune to the effects of their own venom (Cooke and Loeb 1913; Brown and Lowe 1954).

In mammals, major effects include a rapid reduction in carotid blood flow followed by a marked fall in blood pressure, respiratory irregularities, tachycardia and other cardiac anomalies, as well as hypothermia, edema and internal hemorrhage in the gastrointestinal tract, lungs, eyes, liver, and kidneys (table 4). Common symptoms in dogs and cats include vomiting accompanied by discharge of urine and feces, and copious flow of saliva and tears. Death from large doses of *Heloderma* venom has been attributed primarily to respiratory disturbances (Cooke and Loeb 1913). Postmortem examinations reveal congestion and edema in the lungs, a marked congestion of the serous layer of the stomach and intestines, and hemorrhage in the kidneys and liver (Cooke and Loeb 1913; Ariano Sánchez 2003). Sublethal doses in mice and rats produce protrusion of the eyes and periorbital bleeding (Cooke and Loeb 1913; Ariano Sánchez 2003), and in rabbits they lead to an increase in the number of white blood cells (Meyers and Tuttle 1913). In humans, the effects of bites are associated with excruciating pain that may extend well beyond the area bitten and persist up to 24 hours. Other common effects of *Heloderma* bites on humans include local edema (swelling), weakness, sweating, and a rapid fall in blood pressure (see below). A summary of the effects of *Heloderma* venom on mammals is given in table 4.

The lethal dose (LD_{50}) of *Heloderma* venom varies among studies and venom lots (Russell and Bogert 1981). Such values are influenced by the relative amounts of venom and saliva collected in each sample and are, therefore, difficult to evaluate. The venom is most toxic when administered intravenously in mice, with LD_{50} values varying from 0.4 to 2.7 mg/kg for *H. suspectum* (table 5). With an i.v. LD_{50} of 1.4 to 2.7 mg/kg, the venom of *H. horridum* appears to have toxicity similar to that of *H. suspectum*. The LD for *H. horridum charlesbogerti* venom is 1.0 mg/kg when injected intramuscularly in rats (Ariano Sánchez 2003). When injected into mammals, the venom of *Heloderma* appears to be about as toxic as that of the Western Diamondback Rattlesnake, *Crotalus atrox* (Russell and Bogert 1981).

CHEMICAL MAKEUP OF THE VENOM

As knowledge of the toxic effects produced by *Heloderma* venom increased, so did interest in

TABLE 4

Effects of Venom of Helodermatid Lizards in Nonhuman Mammals

EFFECTS	REFERENCES
Respiration	
Initial increase in rate, "forced" respirations followed by decrease and eventual standstill	Cooke and Loeb 1913
Ventilatory irregularities: gasping, rapid, shallow breaths, apnea	Patterson 1967a
Cardiovascular System	
Marked and rapid fall in blood pressure	Mitchell and Reichert 1883
Initial tachycardia	Van Denburgh and Wight 1900
Cardiac irregularities	Fleisher 1913
Impaired cardiac function/cardiac failure	Patterson 1967a
Reduction in carotid blood flow and arterial blood oxygenation	Russell and Bogert 1981
Cyanosis	
Hemorrhage	
GI tract	Cooke and Loeb 1913
Intestines, kidneys, lungs	Patterson 1967a
Eyes	Stýblová and Kornalik 1967
Liver	Ariano Sánchez 2003
Blood	
Leucocytosis	Meyers and Tuttle 1913
No effect on blood coagulation time	Cooke and Loeb 1913
Prolonged prothrombin time (anticoagulation)	Patterson and Lee 1969
Smooth Muscle	
Stimulating effect in ileum, colon, uterus	Patterson 1967b
Edema (note also with human envenomation)	
Intestines, stomach, lungs	Cooke and Loeb 1913
Other Effects	
Paralysis of limbs, partial paralysis of body	Patterson 1967a
Protrusion of eyes (exophthalmia)	Cooke and Loeb 1913
Lacrimation	
Abdominal rigidity	
Convulsions	
Vomiting	

NOTE: Based on in vivo studies in cats, dogs, guinea pigs, mice, and rats.

the chemical constituents causing these symptoms. The first studies on the chemical nature of *Heloderma* venom (Santesson 1897) identified two "toxic principles," referred to as *nuclein* and *albuminose*, but it was not until 1913 that the first toxin, a lipase, was isolated (Alsberg 1913). The 1960s saw renewed interest in the chemistry of *Heloderma* venom. Serotonin and amine oxidase activity were identified in the venom of *H. horridum* in 1960 (Zarafonetis and Kalas 1960). Mebs and Raudonat (1966) identified a very active hyaluronidase (a spreading factor; see below), phospholipase A, and a kinin-releasing enzyme with small proteolytic activity in both *H. horridum* and *H. suspectum* venom. In 1967, Tu and Murdoch showed that *H. suspectum* venom was largely a mixture of proteins, some of which hydrolyze

TABLE 5
LD_{50} of Heloderma *Venom in Mice and Rats*

	AVENUE	SPECIES	LD_{50} (MG/KG)	SPECIES
Johnson et al. 1966	Intraperitoneal	mice	3.0	*suspectum*
Mebs and Raudonat 1966	Subcutaneous	mice	0.82	*suspectum*
Stahnke 1966	Intraperitoneal	rats	10.3	*suspectum*
Stahnke 1966	Intraperitoneal	mice	3.0	*suspectum*
Patterson 1967a	Intracardiac	rats	1.35	*suspectum*
Stýblová and Kornalik 1967	Intravenous	mice	0.4	*suspectum*
Stýblová and Kornalik 1967	Subcutaneous	mice	4.0	*suspectum*
Tu and Murdock 1967	Subcutaneous	mice	2.0	*suspectum*
Stahnke et al. 1970	Subcutaneous	rats	14.0–16.8	*suspectum*
Hendon and Tu 1981	Intravenous	mice	2.7	*suspectum*
Russell and Bogert 1981	Intravenous	mice	0.52	*suspectum*
Mebs and Raudonat 1966	Subcutaneous	mice	1.4	*horridum*
Hendon and Tu 1981	Intravenous	mice	2.7	*horridum*
Alagón et al. 1982	Intraperitoneal	mice	2.0	*horridum*
Ariano-Sánchez 2003	Subcutaneous	rats	1.0	*horridum charlesbogerti*

certain peptides. Patterson and Lee (1969) later showed coagulation was affected if venom was incubated with blood plasma for longer periods. Subsequent work isolated hemorrhagic toxins in the venom (Nikai et al. 1988; see below). In the late 1960s, Mebs isolated kallikrein from the venom of *H. suspectum* (Mebs 1968, 1969). Murphy et al. (1976) demonstrated enzyme activities in the venom of *H. horridum*. In the 1980s, additional proteins—gilatoxin (Hendon and Tu 1981), horridum toxin (an arginine ester hydrolase; Alagón et al. 1982; Nikai et al. 1988), helodermatine (a kallikrein-like hypotensive enzyme; Alagón et al. 1986), an additional phospholipase (A_2; Gomez et al. 1989), and helothermine (Mochca-Morales et al. 1990)—were discovered and added to the known arsenal of *Heloderma* venom components.

With the discovery in the 1980s that *Heloderma* venom contained strongly bioactive agents, with hormonelike actions similar to vasoactive intestinal peptides (VIP), interest and research in these intriguing molecules accelerated dramatically (Raufman 1996). Five bioactive peptides have been isolated so far from the venoms of Gila Monsters and Beaded Lizards: helospectin I and II (Parker et al. 1984), helodermin (Hoshino et al. 1984), exendin-3 (found in *H. horridum;* Eng et al. 1990), and exendin-4 (found in *H. suspectum;* Eng et al. 1992). These peptides mimic several human neurosecretory hormones that relax smooth muscle and mediate energy metabolism. The major compounds so far identified in the venom of helodermatid lizards as well as their chemical nature and physiological effects are discussed below and summarized in table 6.

HYALURONIDASE

Hyaluronidase is a hydrolase enzyme that cleaves internal glycosidic bonds of hyaluronic acid, a mucopolysaccharide that is an important component of connective tissue. Because this action facilitates venom diffusion into the tissue (Tu and Hendon 1983), hyaluronidase has been termed *spreading factor*. Hyaluronidase is also common in snake venoms where it also acts as a spreading factor (Meier and Stocker 1995). Venom of both *Heloderma* species shows particularly high hyaluronidase activity, which is believed to explain the potent edema effects of

TABLE 6

Major Constituents of Heloderma *Venom and Their Actions*

	DESCRIPTION/ACTION	PHYSIOLOGICAL EFFECTS
Hyaluronidase	Hydrolase enzyme; cleaves hyaluronic acid	Acts as a "spreading factor" by facilitating diffusion of venom through connective tissues surrounding bite site
Serotonin	Neurotransmitter hormone	Mediates inflammation, vasodilation, smooth muscle activity, and other effects
Phospholipase A_2	Hydrolase enzyme; catalyze hydrolysis of phospholipid glycerol backbone	Presynaptic membrane toxins in snakes; effects of *Heloderma* phospholipase A_2s are unknown
Nerve Growth Factor	Induce nerve growth; degranulate mast cells	Effects unknown
Helothermine	25-kDa peptide with similarity to family of mammalian cysteine-rich secretory proteins	Causes lethargy, partial paralysis of rear limbs, intestinal distension, and hypothermia in rats
Gilatoxin	33-kDa serine protease glycoprotein	Kallikrein-like lethal toxin; causes lowered blood pressure and contraction of isolated uterus smooth muscle in rats
Horridum toxin	31-kDa glycoprotein similar to gilatoxin	Kallikrein-like lethal toxin; causes hypotension, hemorrhage in internal organs, hemorrhage and bulging of the eyes
Helodermatine	63-kDa serine protease glycoprotein	Kallikrein-like enzyme; causes a dose-dependent decrease in arterial blood pressure in rats
Unnamed lethal toxin	28-kDa peptide	Kallikrein-like lethal toxin that suppresses contraction of isolated diaphragm muscle in mice
Helospectin I & II (exendin-1)	37- to 38-amino-acid peptides from **ex**ocrine gland having **end**ocr**in**e function	Stimulate amylase release from the pancreas; show physiological effects similar to VIP (vasoactive intestinal peptide); have been localized in various human tissues
Helodermin (exendin-2)	Basic 35-amino-acid peptide with stable secondary structure	Causes hypotension in dogs and rats; shows physiological effects similar to VIP
Exendin-3	39-amino-acid (4.2-kDa) peptide from *H. horridum* venom	Interacts with newly described exendin receptor and mammalian VIP receptors; induces amylase release from the pancreas
Exendin-4 (exenatide)	39-amino-acid peptide from *H. suspectum* venom	Induces insulin release through activation of glucagon-like peptide-1 (GLP-1) receptor
Gilatide	Fragment of exendin-4 peptide	Acts on GLP-1 receptor; improves memory in rodents

NOTE: See text for more complete descriptions and references.

Heloderma bites (Mebs and Raudonat 1966; Russell and Bogert 1981).

SEROTONIN

Serotonin, derived from the amino acid tryptophan, produces potent local physiological effects and also functions as a neurotransmitter. Serotonin mediates local processes such as inflammation, vasodilation, and smooth muscle activity and causes aggregation of platelets (Greger 1996). It is found in a variety of toxins from animals such as spiders, toads, and wasps. The specific in vivo effects from the serotonin found in *Heloderma* venom are not known, although it likely participates in the inflammation response.

PHOSPHOLIPASE A_2

Phospholipase A_2 enzymes are classified as hydrolases that act on ester bonds in fat molecules (phospholipids). These common constituents of viperid and elapid snake venoms are toxic to presynaptic membranes, disrupting the release of neurotransmitters at nerve synapses and neuromuscular junctions and inhibiting platelets (among other actions; Rosenberg 1988; Meier and Stocker 1995). This behavior can cause paralysis and loss of muscle control and function, among other signs. We do not know whether phospholipase A_2 from *Heloderma* is responsible for such actions in humans and other mammals because the specific in vivo effects of this enzyme have not been investigated. However, phospholipase A_2 enzymes from *H. horridum* have been shown to inhibit platelet aggregation in human blood plasma (Huang and Chiang 1994).

Five variants of phospholipase A_2 (or PLA_2) have been identified and characterized in the venom of *H. suspectum* (Sosa et al. 1986; Gomez et al. 1989; Vandermeers et al. 1991). All of these stimulate the release of amylase from secretory cells (acini) in rat pancreatic tissue, and they hydrolyze phosphatidylcholines (lecithins), which are important in cell structure and metabolism. Gila Monster PLA_2s are apparently quite different from those found in snake venoms, especially in their N-terminal amino acid sequences and molecular weight (Tu 1991; Vandermeers et al. 1991). Interestingly, they are most similar to PLA_2 from bee venom. The major variant of PLA_2 in Gila Monsters (Pa5) shows a C-terminal extension seen only in *Heloderma* and bee *(Apis mellifera)* venoms (Gomez et al. 1989).

NERVE GROWTH FACTOR

Nerve growth factors are found in a number of viperid and elapid snake venoms. They normally induce the growth of nerve tissues, but they may also contribute to the toxic action of venoms by degranulating mast cells, which contributes to inflammation (Meier and Stocker 1995). A nerve growth factor was discovered in the venom of *H. horridum* in 1968 (Levi-Montalcini and Angeletti 1968), but its role remains unexplored.

HELOTHERMINE

This toxin from *H. horridum* venom is a 25.5 kDa protein with an N-terminal amino acid sequence initially thought to be unique (Mochca-Morales et al. 1990). Further characterization of helothermine showed it to have structural homology with a family of cysteine-rich secretory proteins found in mammalian male genital tracts and in salivary glands, as well as in some other animal toxins (Morrissette et al. 1995; R. L. Brown et al. 1999). When injected into mice, helothermine causes lethargy, partial paralysis of rear limbs, intestinal distension, and lowering of body temperature; hence, as its name suggests, it is a potentially hypothermic toxin (Mochca-Morales et al. 1990). Although deadly to mice, its LD_{50} has not been reported (Mochca-Morales et al. 1990). Helothermine blocks certain ion channels in cell membranes (e.g., calcium channels in cardiac muscle, skeletal muscle, and cerebellar granules; Morrissette et al. 1995; Nobile et al. 1994, 1996). This property gives it promise as a pharmacological tool for studies on the structure and function of ion channels in muscle and brain tissue. Unlike other toxins found in the venoms of helodermatid lizards (discussed below), helothermine

shows no enzymatic effects. Helothermine is a member of the helveprins (20–25 kDa proteins), which are found in many reptile venoms (S. P. Mackessy, pers. comm.).

THE KALLIKREIN-LIKE TOXINS

These are the toxins most responsible for the excruciating pain that results from the bites of Gila Monsters and Beaded Lizards. Four different proteins that possess kallikrein-like toxins have been identified in the venom of helodermatid lizards (table 6). Kallikreins are hypotensive hormones that exert powerful local physiological effects. They cleave kinogens that, in turn, release bradykinins—local hormones that mediate the inflammation/pain response. Bradykinins produce pain by stimulating both sensory C-fibers and spinal ganglia, and they reduce blood sugar by shifting D-glucose from plasma to muscle (Greger 1996). Bradykinins also cause vasodilation of peripheral arterioles and increased vascular permeability, which results in edema (swelling caused by leaking of plasma fluid into tissues). Bradykinins also stimulate secretion of adrenaline, which can cause an increase in heart rate, among other effects. Plasma concentrations of bradykinins are usually very low (4 pmol/L) so local increases in these substances can have dramatic effects (Greger 1996). Kallikreins in venoms of viperids may contribute to the immobilization of prey animals (Meier and Stocker 1995).

As with helothermine, above, the first three kallikrein-like proteins have been shown to be lethal toxins.

GILATOXIN

Gilatoxin, a glycoprotein, was the first lethal toxin isolated from the venom of helodermatid lizards (Hendon and Tu 1981). Later research found that gilatoxin is one of several toxins in *Heloderma* venom that shows kallikrein-like activity (Utaisincharoen et al. 1993). Present in both species, it comprises about 3%–5% of the venom volume. Gilatoxin is a serine protease that acts on a number of substrates including kininogen and angiotensin, which are involved in mediating blood pressure. Gilatoxin appears to be found only in helodermatid lizards and has an LD_{50} of 2.6–2.9 μg/g. Interestingly, it becomes more toxic when administered to mice in combination with other venom fractions (Tu 1991), suggesting that the toxin acts synergistically with additional venom components.

HORRIDUM TOXIN

Horridum toxin, from the venom of *H. horridum*, is the only hemorrhagic toxin so far isolated from helodermatid lizards. Another glycoprotein, horridum toxin is a distinct form of gilatoxin, but with a similar structure (Nikai et al. 1988; Datta and Tu 1997; Tu 2000). It also shows kallikrein-like activity, releasing bradykinin upon hydrolysis of kinogen (Datta and Tu 1997). Similar to gilatoxin, it has strong hypotensive effects when injected into rats. It is more toxic than gilatoxin, showing an LD_{50} of 0.38 μg/g (Nikai et al. 1988). In addition to the inflammatory effects produced by bradykinin, horridum toxin causes hemorrhage in internal organs and especially in the eye, leading to exophthalmia (protrusion of the eyeballs), an effect that has not been observed from other venoms (Datta and Tu 1997). The actual exophthalmic effect, however, occurs only when the kallikrein activity is contaminated with a similar-sized metalloprotease (S. P. Mackessy, pers. comm.). Although horridum toxin has been isolated only from *H. horridum*, exophthalmia has also been observed in rats and mice injected with venom from *H. suspectum* (Cooke and Loeb 1913; Patterson 1967a; Stýblová and Kornalik 1967), suggesting a similar toxin may also be present in the venom of the Gila Monster. Horridum toxin has been shown to degrade fibrinogen to fibrin, an important step in the blood coagulation process that is normally performed by the enzyme thrombin. Clots do not form from horridum toxin poisoning, however, suggesting that it does not act like thrombin, as do many snake venoms that show kallikrein-like activity.

UNNAMED LETHAL TOXIN

Another very toxic "lethal toxin" was isolated from the venom of *H. horridum* by Komori et al. (1988). This toxin has a molecular weight of 28 kDa and an LD_{50} of 0.135 μg/g when injected intravenously in mice. This appears to be the most toxic substance isolated so far from the venom of *Heloderma*. The toxin suppresses contraction of the diaphragm muscle in mice, but shows no hemolytic or hemorrhagic activity. Proteolytic, phospholipase A_2, or arginine ester enzymatic activity are all absent in this apparently novel toxin (Kamori et al. 1988).

HELODERMATINE

Not considered a lethal factor, helodermatine is an arginine esterase purified from *H. horridum* venom. It was the first enzyme with kallikrein-like activity to be isolated and characterized from *Heloderma* venom (Alagón et al. 1986). Helodermatine is another serine protease glycoprotein, as are gilatoxin and horridum toxin, but, at 66 kDa, it is about twice their molecular mass. When injected into anesthetized rabbits, it causes a rapid dose-dependent decrease in arterial blood pressure, an effect likely attributed to its active kallikrein nature. Its N-terminal amino acid sequence is similar to kallikrein from pig pancreas and to kallikrein-like enzymes from venom of the rattlesnakes *Crotalus atrox* and *C. adamanteus*.

THE BIOACTIVE PEPTIDES

In the early 1980s, investigators searching for biologically active chemicals in animal venoms made a very important discovery. They found that the venom of helodermatid lizards elicited a spectacular secretory response from pancreatic acini (secretory cells that make a useful model system for testing the biological activity of chemical substances; Raufman 1996). This discovery led to an intense effort to find the compounds responsible for this response, which culminated in 1984 with the description of helospectin I and II (Parker et al. 1984) and helodermin (Hoshino et al. 1984). These peptides are similar in structure and action to VIP (vasoactive intestinal peptide), a hormone secreted by nerves found throughout the gastrointestinal tract. A neurotransmitter hormone, vasoactive intestinal peptide is a powerful relaxant of smooth muscle (hence its name) and mediates the secretion of water and electrolytes by the small and large intestines. (Interestingly, VIP-secreting tumors give rise to "pancreatic cholera" with symptoms similar to those shown by a severe bite case—see below.) The actions and structure of VIP are also similar to secretin, another messenger peptide involved in stimulating pancreatic secretions. In the early 1990s, two additional biologically active peptides, exendin-3 and exendin-4, were discovered in the venom of helodermatid lizards (Eng et al. 1990, 1992). Exendins are so named because they are peptides from *ex*ocrine glands (of *Heloderma*) having *end*ocrine actions (Eng et al. 1990). The helospectins became known as exendin-1 and helodermin as exendin-2.

Exendins (including helospectins and helodermin) exert their biological effects by interacting with specific receptors found on cell membranes in various tissues of mammals. The receptors known to be involved include VIP and secretin receptors, exendin receptors (newly discovered because of work with *Heloderma* venom), and GLP-1 receptors (involved in insulin release and glucose metabolism). The effects of these bioactive peptides are numerous and diverse, but they generally involve decrease of blood flow via effects on smooth muscle, and activation of adenyl cyclase. Adenyl cyclase, an enzyme released when a mammal requires energy, catalyzes a reaction that results in the formation of cAMP, which, in turn, activates numerous enzymes including those that metabolize carbohydrates for energy production. An excellent review of bioactive peptides from *Heloderma* is given in Raufman (1996). The nature and action of these interesting peptides are discussed below.

THE HELOSPECTINS

Helospectin I and II are very similar peptides (38 and 37 amino acids, respectively) that

stimulate amylase release from the pancreas. Helospectin I differs from helospectin II in that it has an additional serine residue at its C terminus. It is believed that, because of their similar actions, helospectins and VIP act on a common membrane receptor in mammals (Grundemar and Hogestatt 1990). Six forms of helospectin have recently been identified from *H. horridum* venom that show minor differences in the side groups attached to their Ser32 residues (Vandermeers-Piret et al. 2000). Interestingly, helospectins (and helodermin) have been immunohistochemically localized, usually associated with nerve endings, in a variety of human tissues ranging from the upper respiratory tract to the genitalia (Graf et al. 1995; Hauser-Kronberger et al. 1996, 1999). In these tissues, they appear in association with VIP receptors and (as with VIP and secretin) may play a role in regulating secretory activities and local blood flow. The location of these lizard venom peptide sequences within the tissues of mammals leads to some intriguing ecological and evolutionary questions (see below). The helospectins are present in venoms of both *H. horridum* and *H. suspectum*, but they are more abundant in *H. horridum* venom.

HELODERMIN

Helodermin, a basic 35-amino-acid peptide discovered in Gila Monster venom, was the first secretin/VIP-related peptide found in an animal other than a mammal or bird (Hoshino et al. 1984; Vandermeers et al. 1984). In dogs, it causes prolonged, systemic hypotension (Robberecht et al. 1988); in rats, it produces a dose-dependent hypotension, apparently via K^+ channels that exist on arterial smooth muscle cells (Horikawa et al. 1998). The discovery of helodermin also included the discovery of another bioactive peptide, pancreatic secretory factor, that later turned out to be a member of the phospholipase A_2 family (Dehaye et al. 1984; Vandermeers et al. 1984). In comparison with other members of the secretin/VIP family of peptides, helodermin has an unusually stable secondary structure, partly owing to a secondary configuration it maintains in water, which may account for its prolonged physiological action (Blankenfeldt et al. 1996). Helodermin shows 85% identity with helospectin I and II, which probably explains the similarity in their biological activity (Raufman 1996) and also suggests a common evolutionary origin for these venom peptides. Helodermin binds to receptors in a number of human tissues in the gastrointestinal tract, lungs, and even on human breast cancer cells (Raufman 1996). It has also been shown to inhibit growth and multiplication of lung cancer cells (Maruno and Said 1993). Helodermin may also inhibit the activity of phospholipase A_2, suggesting that it could serve a protective function to attenuate phospholipase activation within the venom glands (Raufman 1996). Helodermin is found only in the venom of *H. suspectum*.

EXENDIN-3 AND EXENDIN-4

The most important peptides in *Heloderma* venom, in terms of pharmacological applications, are exendin-3 and exendin-4. Exendin-3, a 39-amino-acid peptide, occurs in the venom of *H. horridum*. It shows similarity with other hormones involved in digestion: glucagon (48%) and human glucagon-like peptide-1 (GLP-1; 50%). In its 12 amino acid residues, it has strong homology with secretin (91%), but only 41% with secretin overall, and only 29% similarity to VIP. It shows limited structural similarity with helospectin and helodermin (32% and 26%, respectively; Eng et al. 1990).

Exendin-4, found only in Gila Monster venom, differs from exendin-3 by two amino acids, suggesting minor evolutionary changes from an ancestral peptide (Raufman 1996). Exendin-4 shows great structural homology (53%) to human glucagon-like peptide-1, a hormone released from the gut in response to a meal (Goke et al. 1993; Raufman 1996; Drucker 2001). GLP-1 stimulates insulin release and moderates blood glucose levels, traits that have attracted interest in its potential for treating Type II (noninsulin dependent) diabetes (Goke et al. 1993; Doyle and Egan 2001). In the blood,

GLP-1 has a short half-life and must, therefore, be administered frequently to maintain adequate insulin levels. Exendin-4, on the other hand, activates the same receptor as GLP-1 but turns out to be more effective at inducing insulin release. Moreover, exendin-4 has a much longer biological action than GLP-1 (Young et al. 1999a; Doyle and Egan 2001). Amylin Pharmaceuticals, Inc., has developed a synthetic exendin-4 (AC 2993) that lowers plasma glucose to within the normal range, without inducing hypoglycemia, in people with Type II diabetes (Kolterman et al. 1999, 2003). In healthy volunteers, exendin-4 also reduces plasma glucose by delaying gastric emptying and by reducing calorie intake (Edwards et al. 2001).

These traits have propelled exendin-4 to the forefront of pharmacological research on the treatment of diabetes. A search of the medical literature for exendins will turn up hundreds of papers published since their discovery in 1990. Adult-onset (Type II) diabetes accounts for most of the 17 million cases of diabetes in the United States. Prospects for exendin-4, or its trade name exenatide (AC 2993), currently in phase 3 development for use in treating human diabetes, are so bright that Eli Lilly & Company closed a $325 million deal in September 2002 with Amylin Pharmaceuticals for rights to develop and market a synthetic version of this compound (fig. 12).

GILATIDE

This novel nine-amino-acid peptide, discovered in 2001, is really a fragment of the larger exendin-4 molecule (Haile et al. 2001, 2002). The extent to which it occurs independently in the venom of helodermatid lizards is not yet

FIGURE 12. The venom of helodermatid lizards also contains several bioactive peptides that have brought the shy, venomous lizards into pharmacology journals and headlines in medicine. The best-known lizard peptide, exendin-4, mediates insulin release and glucose uptake from the blood after a meal. A synthetic version of exendin-4 (exenatide) is a leading candidate for treating adult-onset (Type II) diabetes, which accounts for most of the 17 million cases of diabetes in the United States. Prospects for this drug are so bright that Eli Lilly & Company recently closed a $325 million deal with Amylin Pharmaceuticals for rights to develop and market this compound (photo by Tom Wiewandt).

known. Gilatide has been shown to strongly enhance memory in mice, based on standard cognition tests. This effect appears to be mediated through GLP-1 receptors, a pathway previously unknown to be involved in learning and memory (Haile et al. 2002). Anonyx, a New York–based pharmaceutical company, is investigating the potential for using gilatide to help people suffering from memory disorders such as those associated with Alzheimer's disease and attention deficit/hyperactivity disorder (ADHD). Should gilatide indeed turn out to be an active, independent component of helodermatid lizard venom, what could be a more effective formula for a defensive venom than to mix components producing great pain with one that also enhances one's memory of the experience?

A summary of the major constituents of *Heloderma* venom is provided in table 6. Many of these peptides have valuable research and pharmacological applications. Venom constituents have enabled discovery of new membrane receptors in mammals, have enlightened physiologists as to how these receptors function, and hold promise for treating diseases such as cancer and diabetes. The venom of helodermatid lizards may be unique among reptile venoms in showing such a high number of bioactive peptides (Bertanccini 1976; Raufman 1996). Why do several venom constituents closely resemble human neurosecretory hormones? Why does the venom exhibit a redundancy of peptides producing strong kallikrein-like activity? What is the ecological function of this complex slurry of peptides, and how do the various venom components act to achieve their ecological role, if any? The answers to these questions are best considered in an ecological and evolutionary context.

ECOLOGICAL/EVOLUTIONARY ROLE OF HELODERMATID VENOMS

Although the biochemistry of *Heloderma* venom shows great promise in pharmacology, its ecological role has received less attention. A perennial source of confusion has been whether the venom system of *Heloderma* is used primarily for defense, or to subdue and aid in digesting prey, as is the case for most venomous snakes. In snakes, venom serves an important feeding role, primarily in subduing or immobilizing prey that are large, dangerous, or otherwise difficult to handle (Greene 1997). Most vipers immobilize their prey by rapidly injecting large quantities of toxins that induce hypotension, clotting, and the enzymatic breakdown of tissues, especially the lining of blood vessels (Meier and Stocker 1995; Greene 1997). Many elapid snakes (e.g., cobras and mambas) inject smaller quantities of neurotoxins that immobilize prey by blocking impulses at the neuromuscular junction, paralyzing muscles of respiration and leading to respiratory failure (Greene 1997). Many snake venoms have both tissue-destructive and neurotoxic properties. A secondary feeding role of snake venoms may be to aid in digesting prey, although few studies have directly demonstrated this effect (Thomas and Pough 1979). Predigestion may be particularly important for viperids that inhabit cooler temperate latitudes and feed on relatively large prey (Thomas and Pough 1979; Mackessy 1988; Greene 1997). In contrast to most venomous snakes, effects of envenomation by *Heloderma* tend to be more localized and, as summarized above, show relatively little tissue destruction.

Thus the utility of snake venoms for defense, although often profound, is secondary to their feeding role (Greene 1997). For helodermatid lizards, on the other hand, an elaborate venom system seems unnecessary for subduing prey. Beaded Lizards and Gila Monsters are specialized nest predators that feed almost entirely on eggs or juvenile birds and mammals (see chap. 7). Venom is not needed to subdue defenseless young or eggs in vertebrate nests. Helodermatid lizards do not feed on rapidly moving prey, and, although meals may be large, individual prey tend to be smaller than 10% of their adult body mass. The venom system of *Heloderma* precludes rapid injecting of venom during brief contact, a trait that is important for subduing large prey that are dangerous and

difficult to handle. Moreover, the venom of helodermatid lizards does not appear to have sufficient tissue-destroying properties to be effective in helping to predigest prey (see above).

Nor do limited field observations suggest the use of venom to subdue prey. Wild Gila Monsters feeding on juvenile cottontails (relatively large prey at approximately 45 g each) delicately swallow the nestlings without the characteristic chewing or pumping motions shown when envenomating an adversary (Beck 1990; chap. 7). In captivity, however, I have observed Gila Monsters and Beaded Lizards that were feeding on dead mice "chewing" their prey before swallowing them. Although these observations do not rule out a feeding function, they suggest that the venom of helodermatid lizards does not serve a major role in subduing prey. In some cases, however, especially for juveniles, it could aid in immobilizing prey.

A digestive role of the venom might be ruled out entirely were it not for exendin-4, which comprises up to 5% of the dry weight of Gila Monster venom (J. Eng, pers. comm.) and has been shown to increase 30-fold in the blood of Gila Monsters immediately after a meal (Young et al. 1999b). This result leaves open the intriguing possibility that exendin-4 could regulate glucose uptake in the gut of helodermatid lizards. Could the venom glands, which are modified salivary glands, serve as a source of hormones exerting endocrine control of carbohydrate metabolism in Gila Monsters and Beaded Lizards? We do not yet know what physiological role, if any, exendin serves in *Heloderma*. It remains to be discovered, or discounted, whether exendin might aid in lizard digestion just as it may help humans cope with diabetes. So, whereas it seems obvious that helodermatid lizards need not use venom to subdue their prey, a role of the venom system in processing prey remains a possibility that needs to be further explored (chap. 10).

For defense, on the other hand, the venom system is crucial. Gila Monsters and Mexican Beaded Lizards spend the vast majority of their time hidden in shelters, yet they occasionally travel considerable distances during infrequent aboveground forays (Beck 1990; Beck and Lowe 1991). These large, slow-moving lizards cannot quickly sprint away from a potential threat, as can most other lizards. Their peak speed of 1.7 km/hour on a treadmill (Beck et al. 1995) contrasts strikingly with some inguanine lizards of similar size, which can attain speeds of over 25 km/hour (Garland 1984). These traits make surface-active *Heloderma* particularly vulnerable to predators. Predation is obviously an important factor, and *Heloderma* occasionally succumb to predators (see chap. 6).

Gila Monsters and Beaded Lizards generally avoid encounters with predators by relying on their secretive habits and cryptic patterns. While radiotracking surface-active Beaded Lizards in Jalisco, I often observed that, when lizards noticed my presence from a distance, they would stop moving and press their body to the ground. As I approached more closely, Beaded Lizards (and Gila Monsters) attempted to escape but could be easily overtaken and captured if they could not quickly flee into a burrow. Thus, when confronted with an adversary during an aboveground foray, the inability of *Heloderma* to swiftly escape necessitates an effective defense.

How effective is the bite of *Heloderma* at repelling potential predators? As an adversary is seized by the jaws of a heloderm, a complex mixture of venom proteins and peptides also seizes physiological control of the intruder. Kallikrein-releasing toxins cause immediate bradykinin release accompanied by severe pain, inflammation, weakness, and a rapid drop in blood pressure. Hyaluronidase enzymes facilitate the spread of venom components through connective tissues surrounding the bite site. Horridum toxin may begin causing internal hemorrhaging; helothermine may begin acting to lower body temperature and induce lethargy.

Meanwhile, the heloderm holds on with bulldog tenacity and continues to chew additional venom into the wound. At this point, all of an intruder's attention must be focused on removing the venomous lizard from the bitten

area. And herein lies another of the paradoxes of helodermatid lizards. The same behavior that so effectively delivers venom to its enemies (and makes them wish they had avoided contact to begin with) puts the lizard at risk of injury as an adversary attempts to remedy its painful situation.

How can this apparent paradox be resolved? Is the effectiveness of *Heloderma*'s venomous bite really undermined by its tenacious behavior? There are very few recorded accounts of direct encounters between *Heloderma* and their natural predators (Sullivan et al. 2002; chap. 6). Of 12 cases of Gila Monster bites on dogs reported in Tucson, Arizona, during 2001–02, two resulted in death of the Gila Monster (unpublished records, Arizona Poison Control Center). In most cases, each dog shook off the Gila Monster without seriously injuring it. From reviewing numerous case histories of *Heloderma* bites on humans (see below), I found only one instance in which the lizard was seriously injured while being extracted. In most cases, the lizard was forcefully pulled or pried off, and a few teeth broken, while attention was focused on the bite injury (and not on the lizard). It is plausible that, when bitten by a *Heloderma*, many natural predators would likewise extract the lizard without killing it. Occasionally, *Heloderma* may also be killed while being removed from an adversary. However, it seems unlikely that, if it survived being bitten, an adversary would eat the *Heloderma* (some of the bioactive peptides present in the venom may even suppress appetite; Szayna et al. 2000; Edwards et al. 2001). It is equally unlikely that an adversary would risk future encounters after a bite from one of these dangerous lizards (a fragment of the exendin peptide even helps improve memory; Haile et al. 2002). A predator, therefore, gains nothing by attacking a *Heloderma,* but it does risk injury, incapacitation, or death. Regardless of whether the *Heloderma* were killed, individuals bitten by Gila Monsters or Beaded Lizards would very likely show lower survivorship and reduced reproductive success. Over evolutionary time, predators learn to recognize and avoid noxious and potentially dangerous prey (S.M. Smith 1977; Lindstrom 2001). To the *Heloderma,* as long as the risk of death does not exceed the benefits received from reduced predation owing to recognition by predators, its defensive strategy would be successful. Because this genus has survived for at least 23 million years, it appears such a strategy has, indeed, been effective.

Whether or not the venom system seems efficient to us, *Heloderma* appears to have used it effectively for a long time. Natural selection does not produce adaptations that are perfect by any means, and half-baked solutions are often all that is possible given evolutionary constraints (Gould 1980). Natural selection is limited by the raw material it has to work with, in this case the morphological constraints imposed by the lizard body plan. Helodermatid lizards do not have agile, elongate bodies that enable them to coil and rapidly strike at a foe (that adaptive zone is the domain of the snakes), so a highly specialized venom apparatus designed for efficient injection of venom through hollow fangs does not make sense for *Heloderma*, given the niche it fills and the evolutionary constraints imposed by its generalized lizard morphology.

Lizards (including *Heloderma*) bite as a final means of defense, and when they do they tend to hang on. Interestingly, Alligator lizards of the genus *Elgaria* (family Anguidae, sister group to the Helodermatidae) bite in a manner similar to helodermatid lizards. Southern Alligator lizards *(Elgaria multicarinata)* readily bite when handled, hang on tenaciously when biting, and exert greater bite pressure (similar to the chewing actions shown by *Heloderma*) as attempts are made to extract them from a bitten extremity (personal observation). For a bite to be effective, a lizard may have no choice but to hang on. If the lizard releases its hold before an adversary has been effectively stunned, surprised, or immobilized (in the case of *Heloderma*), it would be vulnerable to subsequent capture and potential consumption. In this context, it makes sense why *Heloderma* behave as they do when they bite and why they do not possess a more "efficient" venom system. What helodermatids

lack in their "crude" delivery system, however, they compensate for in a sophisticated array of venom constituents and bite tenacity. No doubt, the venom chemistry of *Heloderma* is efficient for defensive purposes.

One might more appropriately ask: Why are there not more lizards that use a venom system for defense? The answer might be that most lizards can run away more effectively than they can bite. However, there are rare cases where bites of varanid lizards may produce symptoms of toxicity (Ballard and Antonio 2001). In at least three cases, reptile keepers bitten by *Varanus griseus*, the Desert Monitor, showed symptoms ranging from dysphagia (difficulty swallowing), tightness of the chest muscles, and dyspnea (labored respiration) to dizziness, facial pain, and muscle soreness (Ballard and Antonio 2001). Desert monitor lizards have shown chewing motions during bites that may continue for over one minute. Whether these signs/symptoms are truly reactions to toxic peptides or proteins in the venom of *Varanus* needs to be thoroughly investigated.

Of the few other vertebrates known to use a venom system exclusively for defense, the Platypus *(Ornithorhynchus anatinus)* and Stonefish *(Synanceja horrida)* are noteworthy. Peptides and other components in Platypus venom relax smooth muscle in rats, promote edema and inflammation, and produce extreme pain (Fenner et al. 1992; De Plater et al. 1998). Stonefish venom produces intense pain and induces hypotension via relaxing smooth muscle in the walls of blood vessels (Low et al. 1993). These venoms act to repel and potentially immobilize potential predators with symptoms (severe pain, inflammation, and hypotension) similar to those produced by bites of *Heloderma*. Spitting cobras seem to have evolved the ultimate venom delivery system for defense. Venom is ejected from the fangs as a jet that becomes a fine spray (Bogert 1993). A tiny speck of African Spitting Cobra, *Naja nigricollis*, venom landing in the eye produces a pain, in the words of a photographer affected by the venom, greater than any ever experienced (Bogert 1993).

In contemporary helodermatid lizards, the venom system functions as an effective defense mechanism, while some of the bioactive peptides in the venom could play a role in digestion. The venom proteins and peptides appear to act primarily on initiating a powerful inflammatory/pain response, relaxing smooth muscle (which results in vasodilation and hypotension), and influencing energy metabolism. Taken together, the venom constituents clearly act physiologically to immobilize or incapacitate the bite victim. Some of these components not only profoundly affect the physiology of potential mammalian adversaries, but they are, in a sense, *part* of their physiology. These venom components are analogues of important mammalian hormones, including serotonin, secretin, VIP, and GLP-1. The kallikrein enzymes are common participants in the inflammatory response in mammals. As noted above, many of the bioactive peptides (helospectin and helodermin, in particular) have been located, using immunohistochemical techniques, in several mammalian tissues including the brain, pancreas, blood, colon, lung, stomach, breast, heart, intestine, uterus, liver, urogenital system, and others (Raufman 1996). In these tissues, they appear in association with membrane receptors that play a role in regulating secretory activities and local blood flow. When they were first discovered, it was thought that some of the bioactive peptides in *Heloderma* venom represented homologues of mammalian hormones yet to be discovered (Parker et al. 1984). This appears not to be the case; for example, helodermin and exendin-4 from *Heloderma* are not the evolutionary precursors to mammalian VIP and GLP-1. They are distinct peptides encoded by different genes and, therefore, appear to have evolved independently of the mammalian peptides they resemble (Chen and Drucker 1997; Pohl and Wank 1998). To find so many *Heloderma* venom peptide sequences within the tissues of mammals suggests an intricate example of biochemical mimicry. The venomous bite of helodermatid lizards has apparently evolved to incapacitate and deter an enemy with a diversity of toxins that

manipulate its physiology in a variety of ways. An intriguing possibility also exists that exendin, and perhaps other bioactive peptides in the venom of Gila Monsters and Beaded Lizards, may act to regulate the lizards' own digestive physiology (Young et al. 1999b). What has previously been called a "primitive," "crude," or "inefficient" venom system appears, in fact, highly refined and effective.

Since the early 1980s, when many of the constituents of *Heloderma* venom were discovered, little attention has been directed at whole-venom physiological effects. When taken as a whole, many of the venom components may show synergistic consequences that are not apparent when examined alone. For example, gilatoxin shows greater toxicity when combined with other venom components than when acting alone (Tu 1991). It is likely that other venom constituents, especially the kinin-releasing peptides and the bioactive peptides, may interact in similar ways. Investigations into how the venom components may interact with one another in mammalian tissues would be another fruitful arena for future research (chap. 10). A picture of whole-venom effects partially emerges by examining the effects of *Heloderma* envenomation in humans, which we consider next.

HUMAN ENVENOMATION BY *HELODERMA:* OVERVIEW OF BITE CASE HISTORIES

A rich, entertaining, and commonly erroneous literature is associated with bite case histories of *Heloderma* (see chap. 1). Vivid tales abound recounting suffering, terror, and death. I have found more exaggerated and erroneous information associated with this *Heloderma* topic than with all others combined. Woodson (1947) provided one of the first summaries of the bite of *Heloderma*, reporting that 29 of 136 cases ended in death. Bogert and Martín del Campo (1956) summarized 34 cases between the 1880s and 1956, mostly from newspaper clippings and correspondence, eight of which allegedly resulted in death. Both Woodson (1947) and Bogert and Martín del Campo (1956) emphasized that details regarding deaths from *Heloderma* bites were often sketchy, contradictory, and invalidated, and that alcohol was involved in most cases. Russell and Bogert (1981) discussed the outcomes of another 16 cases, as well other published records available to them (Shannon 1953; Bogert and Martín del Campo 1956; Grant and Henderson 1957; Albritton et al. 1970; Stahnke et al. 1970). None of the 16 cases mentioned by Russell and Bogert resulted in death. Since the dubious case in 1930 of an intoxicated pool-hall operator in Casa Grande, Arizona (chap. 1), there have been no authenticated reports of a death from the bite of *Heloderma suspectum*. In fact, I have not even been able to find any published validated cases of truly "legitimate" bites (i.e., victims being accidentally bitten by wild Gila Monsters that were not poked, picked up, handled, or otherwise harassed).

Hearsay and fertile imaginations have, therefore, produced far more information about the bite of *Heloderma* than have scientific records and reports. Of the many bite cases reported in newspapers, correspondences, and the popular literature since 1950, I could find only 17 that were based on firsthand information and published in peer-reviewed biological or medical journals (tables 7 and 8). Even in these cases, one description of a myocardial infarction following the bite of a Gila Monster (case no. 11, tables 7 and 8) was published twice, by different authors in 1988 and 1989 (Bou-Abboud and Kardassakis 1988; Preston 1989). A summary of these bites and their signs and symptoms are given in tables 7 and 8. Since 1950, bites by *Heloderma* have become less newsworthy, so only the most serious or noteworthy cases get reported in scientific journals. Since 1981, I could find only 10 such cases. Tables 7 and 8 do not include cases reported in the news media (unless they are also published in a peer-reviewed journal), or those contained in unpublished hospital records, regional reports, etc. Tables 7 and 8 should be interpreted carefully, therefore, because, although they contain some of the best-documented cases, they are not necessarily the most representative.

TABLE 7
Heloderma *Bite Cases Published in Peer-Reviewed Journals since 1950*

CASE NO.	CAUSE (ALL FROM CARELESS HANDLING)	BITE LOCATION	REFERENCES
1	Pet	Finger	Shannon 1953
2	Pet	Finger	Shannon 1953
3	Demonstration	Finger	Tinkham 1956
4	Demonstration	Thumb	Grant and Henderson 1957
5	Demonstration	Finger	Albritton et al. 1970
6	Demonstration	Finger	Stahnke et al. 1970
7	Pet	Hand	Roller 1977
8	Pet	Abdomen	Heitschel 1986
9	Wild capture	Forearm	Piacentine et al. 1986
10	Pet	Hand	Streiffer 1986
11	Wild capture	Forearm	Bou-Abboud and Kardassakis 1988 and Preston 1989
12	Recent capture	Calf	Caravati and Hartsell 1994
13	Wild capture?	Thumb	Caravati and Hartsell 1994
14	Pet	Shoulder	Caravati and Hartsell 1994
15	Wild capture	Triceps	Caravati and Hartsell 1994
16	Pet	Finger	Strimple et al. 1997
17	Pet	Hand	Cantrell 2003

Nevertheless, an analysis of the cases summarized in tables 7 and 8 is instructive. Most bites were to a finger or hand and came from pet Gila Monsters. These bites are more likely to produce intense pain and local edema but are less likely to produce more serious symptoms such as severe hypotension, cardiac and blood abnormalities, or shock. All bites resulted from careless handling, some of which occurred during public demonstrations or classroom lectures. Alcohol was involved in some of the most serious cases (Heitschel 1986; Piacentine et al. 1986; Caravati and Hartsell 1994).

Based on an examination of case histories by Bogert and Martín del Campo (1956), Russell and Bogert (1981), recent bite case reports (tables 7 and 8), and my knowledge of approximately 30 unreported or untreated bites over recent years, the most common signs and symptoms of *Heloderma* envenomation in humans are similar to those observed in other mammals (table 4). They include pain, local edema, and a feeling of weakness, faintness, or nausea. Bleeding at the site of the bite may be profuse, not from anticoagulant effects of the venom, but from the lacerating effect of the teeth and tenacity of the bite (fig. 13). Pain, often severe, usually begins within minutes, and may last several hours. The pain has been described as a steady burning, like a spine imbedded in the flesh. Pain may spread well beyond the site of the bite; a person bitten on the arm may feel pain from the shoulder to the hand. Edema (swelling) can occur within minutes and extend well beyond the region of the bite (fig. 13).

A second set of relatively common symptoms includes hypotension, sweating, an increased heart rate, and vomiting. Blood chemistry changes, including elevated leukocyte count, reduced potassium levels, and reduced platelets, are occasionally shown in moderately severe cases (tables 7 and 8). There is limited evidence that previous exposure to *Heloderma* bites may sensitize some individuals and result in an allergic reaction to the venom (Cantrell 2003). More severe cases seem to result from bites

TABLE 8
Bite Signs/Symptoms and Their Frequency

	PERCENT (%) OF CASES	CASES REPORTING (FROM TABLE 7)
Pain	82	1–3, 5–8, 10–13, 15–17
Local edema/swelling	82	1–7, 9–11, 13, 15–17
Weakness, faintness, dizziness	65	1, 3, 4, 6, 8, 11–13, 15–17
Nausea	65	2, 3, 5–8, 10, 12, 14, 16–17
Hypotension	47	6, 8–14
Diaphoresis (sweating)	47	4, 8–11, 13, 16–17
Tachycardia (elevated heart rate)	35	5, 8, 11, 12, 14, 15
Vomiting	35	3, 5, 10, 12, 14, 17
Leucocytosis (elevated WBC count)	29	6, 8, 11, 12, 17
Hypesthesia (hypersensitivity around bite)	24	1, 2, 12, 16
Reduced blood potassium levels	18	8, 9, 11
Reduced platelets	18	8, 11, 17
Cyanosis (bluish discoloration around bite)	13	3, 8
Cardiac abnormalities	12	7, 11
Swollen or painful lymph glands	12	10, 11
Lethargy	12	8, 14
Anaphylaxis	6–12	9, possibly 17
Diarrhea	6	8
Tinnitis (ringing in ears)	6	2
Exophthalmia or periorbital hemorrhage	6	8
Hypothermia	6	12
Miosis (contraction of pupil)	6	14

located closer to the core of the victim's body, such as the abdomen, shoulder, calf, or forearm (tables 7 and 8). The most severe cases result in extreme hypotension, which may be accompanied by life-threatening anaphylaxis (Piacentine et al. 1986), coagulopathy and acute myocardial infarction (Bou-Abboud and Kardassakis 1988), or profuse diarrhea and lethargy (Heitschel 1986). These cases likely illustrate the powerful physiological effects of the kallikrein-like and bioactive peptides found in *Heloderma* venom.

In the most serious case, a young woman had hidden a Gila Monster under her sweater and walked into a tavern, whereupon the lizard bit her on the abdomen. Her screams alerted bar patrons of her condition, and her boyfriend removed the lizard by severing its head. When she arrived at the hospital, the woman was nearly incapacitated, showing severe pain, vomiting, and diarrhea. She remained in intensive care for 36 hours (Heitschel 1986). This case (the only female in the sample) is noteworthy because the victim's symptoms are similar to pancreatic cholera, a condition produced by VIP-secreting tumors and also known as WDHA (watery diarrhea, hypokalemia, acidosis) syndrome (Connigrave and Young 1996). Such symptoms may be an example of the action of VIP-like bioactive peptides in *Heloderma* venom.

TREATMENT OF BITES

Bites by *Heloderma* may be increasing because, as captive-breeding techniques have improved, more people are keeping these lizards in private collections (chap. 8). The docile nature of *Heloderma* kept in captivity often lulls their handlers into a dangerous habit of complacency. The vast majority of bites by helodermatid lizards occur on the fingers or hands.

FIGURE 13. Bite to the index finger from a *Heloderma suspectum*. The laceration occurred as the Gila Monster was extracted from the finger. Considerable edema that commonly occurs from Gila Monster bites had already subsided when this photograph was taken two days later (photo by M.L. Gilbert).

Most of these pass on uneventfully and go unreported (Miller 1995). Although a *Heloderma* bite is very unlikely to be lethal to a healthy adult, it should nevertheless be considered a serious medical emergency. A common misconception is that only the dentaries in the lower jaw (and to a lesser extent the maxillaries in the upper jaw) can deliver sufficient venom to cause serious effects. This is not the case; significant symptoms can occur by a seemingly minor "slashing" bite from even the premaxillary teeth toward the front of the mouth (Tinkham 1956; Strimple et al. 1997).

When a person is bitten by a *Heloderma,* the following first aid measures are recommended:

1. Remove the lizard as quickly as possible. The longer the lizard bites, the more venom it is able to deposit into the wound and the more likely the bite is to produce serious symptoms. In mild bites, where only a fold of skin is bitten, it may be possible to simply hold the lizard behind the jaws and carefully pull it away; in cases where the jaws are more firmly attached, it may be necessary to pour water on the lizard or to pry it off with pliers or some other device. A thin, flat lever inserted between the lower jaw and the flesh and turned 90 degrees may work to quickly release the jaws. When *Heloderma* are forcefully removed from the bite site, as is often required, teeth are usually pulled out and a laceration results (fig. 13). I do not recommend trying to remove the lizard by applying a flame to its chin or by using dangerous solvents such as gasoline (which have been advocated in the past). These measures only add to the possibility that additional injury and pain will result.
2. Immediately remove any rings, bracelets, or other jewelry (including piercings). These articles may cause complications as edema (swelling) develops.
3. The bitten part should be immobilized; a light cloth bandage and mild pressure may be applied to control any bleeding.
4. The victim should be transported (by another person) to medical care as quickly as possible and reassured that they will not die.
5. DO NOT apply stun guns, heat, or ice to the wound. DO NOT use tourniquets or constriction bands of any kind nor make incisions to suck out venom.

Once the victim has arrived at the hospital, vital signs should be monitored immediately. One of the biggest dangers is shock/hypotensive crisis brought about by a rapid fall in blood pressure. This can be treated in the victim by infusing electrolyte solutions and administering antishock drugs. Pain normally

peaks within 1 to 2 hours, but may linger for days (Caravati and Hartsell 1994). It can be difficult to relieve; analgesics and morphine have been used effectively (Strimple et al. 1997). Edema normally peaks within 2 to 4 hours and resolves itself without special measures within 72 hours. Because it is largely subcutaneous, edema has not been reported to cause compartment syndrome or neurological problems. Depending on the severity of the bite, laboratory blood tests should be performed to assess the possibility of electrolyte imbalance, leukocytosis, and coagulopathy, which have been reported previously (see tables 7 and 8). An electrocardiograph should be used to evaluate any heart anomalies; myocardial conduction disturbance (Roller 1977) and myocardial infarction (Bou-Abboud and Kardassakis 1988) have been observed. Antihistamines or corticosteroids are usually unnecessary because allergic reactions are rare, although one case of anaphylaxis has been reported (Piacentine et al. 1986). The wound should be carefully inspected for any broken teeth and thoroughly cleaned with antiseptic. Soft-tissue radiography is not sufficient to locate broken teeth (Caravati and Hartsell 1994). Antibiotics are routinely given, although tissue necrosis and infections are very rare. Tetanus immunization should be updated if necessary. Most victims of *Heloderma* envenomation are released from the hospital within 24 hours and recover completely within 2 weeks. More severe cases may require hospitalization up to 48 hours (e.g., Heitschel 1986).