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Venom Ontogeny in the Pacific Rattlesnakes *Crotalus viridis helleri* and *C. v. oreganus*

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Ontogenetic variation in venom composition was examined in the Pacific rattlesnakes *Crotalus viridis helleri* and *C. v. oreganus*. Venoms were analyzed for protease, phospholipase A_2, L-amino acid oxidase, exonuclease and elastinolytic activities, and toxicity toward a native prey (*Sceloporus graciosus*). Protease activity increased significantly with size; L-amino acid oxidase and exonuclease activities also tended to increase. Phospholipase A_2 activity decreased significantly with size, as did venom toxicity. These factors produce a highly toxic venom with low protease activity in juvenile snakes, which facilitates efficient handling of lizards and young rodents. Analysis of gut contents of museum specimens showed that lizards constitute a major fraction of prey taken by juvenile rattlesnakes. Lizards continue to be taken with high frequency until snakes reach approx. 500 mm in total length; above this size, mammals are taken exclusively. As snakes increase in size, they feed on larger mammalian prey, and a functionally different venom is produced. Venom from adult Pacific rattlesnakes is less toxic but has high protease activity, aiding in the digestion of prey in a thermally variable environment.

RATTLESNAKE venoms are among the most complex vertebrate oral secretions (Gans and Elliott, 1968), both in terms of chemical composition (Iwanaga and Suzuki, 1979; Tu, 1982) and the pharmacological effects they produce (Russell, 1980). Once regarded as relatively constant in composition for a given species (Bertke et al., 1966), venoms are presently known to vary in composition as a function of geographic distribution (Jiminez-Porras, 1964; Glenn et al., 1983; Minton and Weinstein, 1986), season (Ishii et al., 1970), age (Minton, 1967; Reid and Theakston, 1978; Lomonte et al., 1983), and species (for reviews see Tu, 1977, 1982; Lee, 1979). The many factors influencing venom composition remain incompletely known, and only interspecific variation has been documented in detail.

Differences in venom composition related to snake age have been recorded for a few species, but the functional significance of venom ontogeny is poorly understood. The ultimate reasons why this variation should exist remain virtually unaddressed. The biochemical/pharmacological and the evolutionary/ecological literatures on snake venoms have largely traveled separate tracks. However, an integration of these disciplines is necessary to address adequately the questions of what chemical variations in composition occur, what functional roles various components have in prey capture/manipulation, and what significance venoms have to the evolution and ecology of venomous snakes.

The present study describes ontogenetic changes in venom composition in *Crotalus viridis*, correlates these to changes in diet, and relates them to the biological roles of venoms. Selective advantages favoring the evolution of venoms with the biological roles of rapid prey immobilization and predigestive action (not necessarily mutually compatible functions) are discussed.

MATERIALS AND METHODS

Specimens and venom samples.—Seventy-four individuals of all size classes of *Crotalus viridis helleri* and *C. v. oreganus* were collected from a variety of localities in Santa Barbara, San Luis Obispo, and Ventura counties in southwestern California between March 1980 and June 1984; both subspecies were approximately equally represented. No differences were noted between the subspecies' venoms in this part of the range, and enzyme assay data were pooled. Samples were extracted once from 25 snakes; 49 snakes 240–600 mm in total length (snout to base of rattle) were maintained in a controlled environment room and milked bimonthly to follow changes in venom composition within in-

dividuals. Specimens are deposited in the Vertebrate Museum, University of California, Santa Barbara.

All venom samples were extracted manually. Venom was collected from snakes <600 mm in length by placing a 100 μl capillary tube over each fang and gently massaging the gland. This method minimized venom loss and allowed estimation of venom volume. Adult snakes were cooled 20 min as a safety precaution; cooling increased the venom yield, as such snakes were less likely to eject venom when pinned for handling. Collected venom was placed in one dram vials, immediately shell-frozen in a dry ice/methanol slurry, and promptly lyophilized. Vials were then capped and stored with desiccant at −20 C until used, ensuring conservation of toxicity and enzymatic activity (Iwanaga and Suzuki, 1979). Hide powder azure was obtained from CalBioChem. All other biochemicals and reagents (analytical grade) were obtained from Sigma Chemical Co.

Gut content analysis.—The stomachs and intestines of preserved rattlesnakes (*C. v. helleri*) housed at the San Diego Society of Natural History Museum (SDSNH) were examined. These snakes were collected from San Luis Obispo Co., California, south to the Mexican border. One hundred thirty-five specimens of 260 examined contained traces of fecal material or partial stomach remains which could be identified as lizard or mammal remains; most samples were excised from the large intestine and consisted of hair or scales. Seventy-eight percent of the specimens containing remains were <500 mm (total length). SDSNH catalog numbers of snakes examined are given in Material Examined.

Enzyme assays.—General protease activity was assayed using hide powder azure (Steyn and Delpierre, 1973) and 20 μg/ml lyophilized venom. The absorbance of the blue solution thus obtained was measured on a Varian 634 spectrophotometer at 595 nm (10 mm path length). A total digest with crude venom of 12 mg substrate in 3.0 ml buffer gave a solution of 1.45 absorbance units at 595 nm and was the basis for expressing venom hydrolysis as a percentage of total substrate hydrolyzed. Blanks (buffer and substrate lacking venom) gave readings of 0.007 ± 0.002 absorbance units (595 nm).

Assays for L-amino acid oxidase activity in crude venoms followed Weissbach et al. (1960) as modified previously (Mackessy, 1985). Blank values for the assay were 0.081 ± 0.006 absorbance units (331 nm).

Assays of crude venom for exonuclease (phosphodiesterase) activity followed Björk (1963) with the following modifications: 0.1 M 2(N-cyclohexylamino) ethanesulfonic acid (CHES)-HCl buffer, pH 8.9, and a total assay volume of 1.5 ml containing 100 μg venom. The reaction was terminated by the addition of 1.5 ml 0.005 N NaOH and absorbance was measured at 400 nm. Blank values were 0.006 ± 0.003 absorbance units (400 nm).

Assays for phospholipase A_2 (phosphatide-2-acylhydrolase: EC 3.1.1.4.) activity were similar to Kawauchi et al. (1971), but utilized a pH-Stat. L-α-phosphatidyl choline, diarachidoyl, was suspended in 0.001 M calcium chloride at a concentration of 1.0 mg/ml by sonication at approx. 7000 cycles/sec for 10 min using a Polytron sonicator (Brinkman Instruments, PCU-2-110). New substrate solutions were prepared for each set of experiments. The reaction was followed for at least 90 sec and the production of arachidonic acid was recorded as μmoles NaOH titrated. Units were expressed as μmoles arachidonic acid liberated/min/mg venom. Incubation of substrate alone did not result in spontaneous release of significant quantities of arachidonic acid during the time interval used.

The method of Simpson and Taylor (1973) was used to assay venom for elastinolytic activity with the following modification: 4.0 mg Congo red-elastin was suspended in a total volume of 4.0 ml 0.05 M tris(hydroxymethyl)methylaminopropane sulfonic acid (TAPS)-HCl buffer, pH 8.8, with 100 μg venom. Absorbance of the filtered solution was read at 495 nm. Control solutions gave readings of 0.013 ± 0.012 absorbance units. Hyaluronidase activity was assayed using the turbidimetric method of DiFerrante (1956). Positive controls utilized sheep testis hyaluronidase.

Venom toxicity toward lizards.—Toxicity of venoms from adult and juvenile *C. v. helleri* and *C. v. oreganus* was evaluated using the sagebrush lizard *Sceloporus graciosus.* All lizards were collected in the vicinity of Alamo Mtn., Ventura Co., California.

Toxicity tests were conducted at dose levels of 10.0, 30.0, 50.0, 70.0, and 90.0 μg venom/g body weight. Eleven lizards were used at each dose level for juvenile and adult venoms within 3 d of capture. Lyophilized venom was recon-

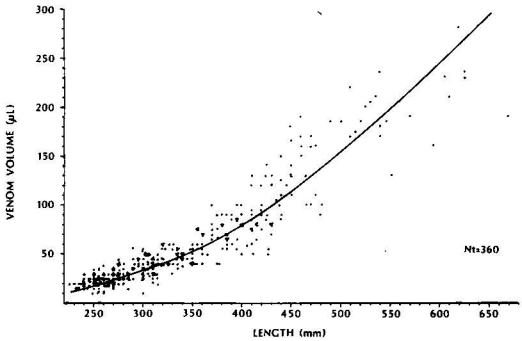

Fig. 1. Relation of snake size to venom volume produced by *Crotalus viridis helleri* and *C. v. oreganus*. Volume of venom increases exponentially with size according to the equation $V = a(TL)^b$ ($r^2 = 0.85$). Length is snout to rattle base.

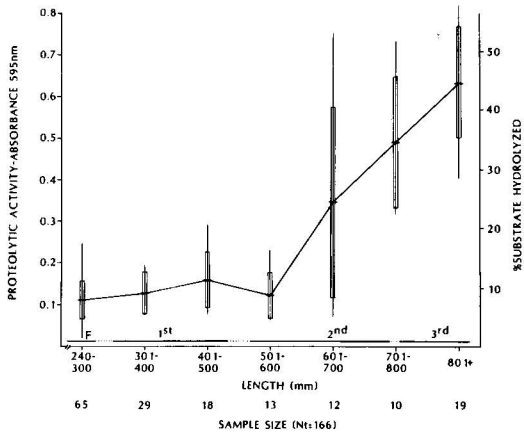

Fig. 2. Proteolytic activity as a function of length in Pacific rattlesnakes. Horizontal lines indicate mean values, vertical lines indicate range of values, and vertical bars represent one standard deviation. The superscripted line indicates approximate age classes (F = first fall, 1st =first complete year, etc.; data from Klauber, 1956, for *Crotalus viridis helleri*).

stituted in 0.9% saline carrier and injected intraperitoneally just anterior to the right hind leg at a depth of 4 mm using Hamilton microliter syringe. Lizard weights were in the range of 3.9–8.2 g. Within an experiment (utilizing 30–50 lizards), weights did not vary by more than 1.5 g, and all doses were adjusted to individual weights. All lizards were kept at 26–27 C and were allowed to equilibrate for 2 h prior to use.

Two measures of toxicity were used: time to incapacitation and time to death. Time to incapacitation was defined as the minimum time from injection to the point a lizard was unable to right itself quickly (within 20 sec). Although lizards showed signs of envenomation well before loss of this reflex, they intensely resisted being placed on their backs and thus this measure provided a reliable measure of major physiologic incapacitation. Death was defined as the lack of a flinch reflex when the hind legs were pinched with forceps. At least one lizard from each dose level was autopsied at 24 h post-injection after remaining at 26 C. Gross structural damage was noted.

TABLE 1. ENZYME ACTIVITIES OF VENOM FROM JUVENILE (<350 MM TL) AND ADULT (>700 MM TL) PACIFIC RATTLESNAKES, *Crotalus viridis helleri* AND *Crotalus v. oreganus*. All activities are expressed as units/min/mg venom. Units: Protease: % substrate hydrolyzed. Phospholipase A_2: µmoles arachidonic acid released. Elastinolytic: ΔA_{495nm}. L-amino acid oxidase: ΔA_{331nm}. Exonuclease: ΔA_{400nm}. J = juvenile; A = adult. *P* based on Student's t-test.

Enzyme assay	N	Size class	Enzyme activity ($\bar{x} \pm SD$)	*P*
Protease	89	J	4.6 ± 1.8	.005
	29	A	23.0 ± 6.0	
Phospholipase A_2	8	J	21.9 ± 3.0	.005
	4	A	4.8 ± 3.2	
Elastinolytic	4	J	0.0019 ± 0.0001	n.s.
	11	A	0.0017 ± 0.0001	
L-amino acid oxidase	12	J	0.247 ± 0.055	.10
	13	A	0.362 ± 0.082	
Exonuclease	8	J	0.064 ± 0.045	.10
	16	A	0.084 ± 0.050	

RESULTS

Qualitative differences.—Venom from adult and juvenile snakes differed in two visual qualities, color and turbidity. Newborn rattlesnake venom was colorless and free of turbidity; as the snakes aged, venom progressively became more yellow and contained more suspended solids. Adult venom was bright yellow and contained large amounts of suspended solids. Venom volume is positively correlated with length of the snake (Fig. 1).

Snakes are able to control the quantity of venom released and to evacuate the left and right glands independently. Gland evacuation does not seem to be an "all or none" response, nor is it a discrete quantal release. In young snakes, venom voluntarily ejected (not manually

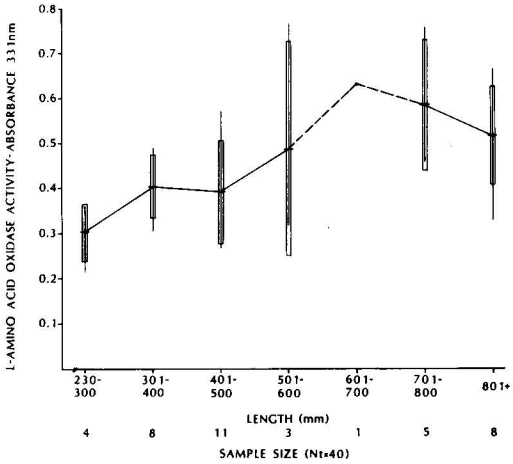

Fig. 3. L-amino acid oxidase as a function of length of Pacific rattlesnakes. Symbols as in Figure 2.

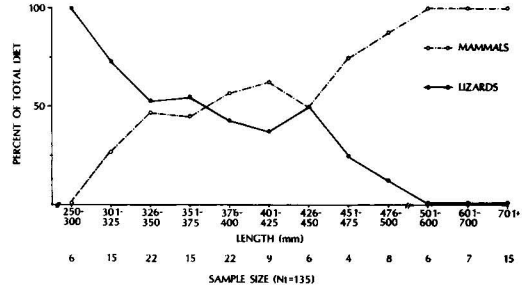

Fig. 4. Prey preference as a function of length of *Crotalus viridis helleri*. Gut contents of preserved specimens were identified as lizard or mammal remains; the curves represent total remains found within a given size class.

extracted by applied pressure) was relatively free of suspended solids while quantities extracted by massaging the gland were much more turbid.

Enzyme activities.—The relation of proteolytic activity to size is shown in Figure 2. In general, proteolytic activity increases with length; however, activity remains low until snakes reach 600–700 mm in length. At this point there is an abrupt increase in activity (approximately three-fold) which continues thereafter with length. A comparison of protease activity of venoms from juvenile and adult snakes is shown in Table 1. Proteolytic activities of juvenile and adult venoms are significantly different. Subspecific variation in venom protease activity was insignificant for snakes collected in the areas mentioned above.

Phospholipase A_2 activity assays show significant differences in activity between juvenile and adult venoms (Table 1). Phospholipase A_2 activity is consistently much higher in juvenile venoms.

The results of elastinolytic activity assays are shown in Table 1. No significant difference in activity occurs, and the elastin-degrading activity is apparently a minor constituent of venoms of *C. v. helleri* and *C. v. oreganus* (Bernick and Simpson, 1976). L-amino acid oxidase activity assay results are shown in Figure 3. Although activity drops off in venoms from large individuals, it tends to increase with length. The activities of venoms from adult and juvenile snakes are shown in Table 1; differences in activity are not significant. The results of exonuclease assays are shown in Table 1; activities are not significantly different. No hyaluronidase activity was detected in either juvenile or adult venoms.

For comparative purposes, venom from adult Colorado desert sidewinders (*C. cerastes laterorepens*) and Mojave rattlesnakes (*C. scutulatus scutulatus*) was assayed for protease activity. Venom of *C. c. laterorepens* showed very high activity, while all samples of venom of *C. s. scutulatus* showed very low proteolytic activity (Table 2). All *C. s. scutulatus* were collected near Lancaster, California, and have type A venom (Glenn and Straight, 1978).

Gut content analysis.—Data from stomach and intestine contents of *C. v. helleri* are shown in Figure 4. Total sample size was 135, with 107

TABLE 2. PROTEASE ACTIVITY OF VENOM FROM SIDEWINDERS (*Crotalus cerastes laterorepens*) AND MOJAVE RATTLESNAKES (*Crotalus s. scutulatus*).

Species	Total length (mm)	% Substrate hydrolyzed/min/mg venom
C. c. laterorepens	509	28.3
C. c. laterorepens	545	26.1
C. c. laterorepens	555	31.1
C. s. scutulatus	510	2.7
C. s. scutulatus	780	1.4
C. s. scutulatus	795	2.6
C. s. scutulatus	810	1.0
C. s. scutulatus	825	0.67
C. s. scutulatus	828	0.33
C. s. scutulatus	840	0.67
C. s. scutulatus	905	2.6
C. s. scutulatus	915	1.0

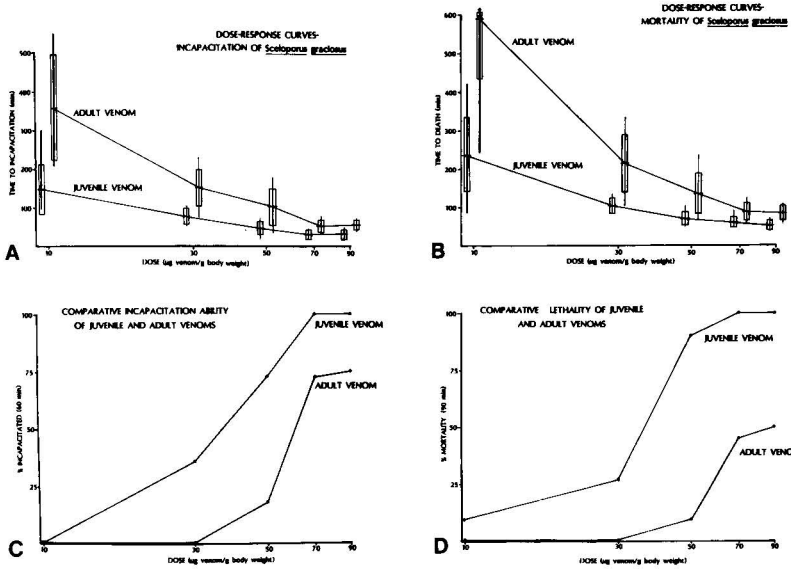

Fig. 5. Toxicity of juvenile and adult Pacific rattlesnake venom to a native prey species (*Sceloporus graciosus*). Dose-response curves are semi-log plots of time to incapacitation (A) and time of death (B) as a function of venom dose administered. Each mean value is based on 11 lizards. Curves in C and D represent the comparative incapacitating and killing abilities of juvenile and adult venoms at 60 and 90 min, respectively.

snakes <500 mm in total length. Lizards are an important component of the diet of young southern Pacific rattlesnakes, comprising 50% or more of prey taken by snakes <450 mm in length. The smallest snakes (<300 mm) contained only lizard remains, although four snakes of this size contained unidentifiable remains which appeared to be insect larvae. For snakes over 500 mm the diet consisted exclusively of mammals.

Toxicity of venoms toward lizards.—The effects of adult and juvenile venoms on *S. graciosus* are plotted in dose-response curves (Fig. 5A–B). These lizards are more sensitive to juvenile than adult venom both in terms of time to incapacitation and time to death. At high dose levels (70–90 μg venom/g body weight), the curves flatten out and approach one another, indicating a maximal response. Although LD_{50} values are not determined, preliminary experiments utilizing 1.0, 10.0, and 100.0 μg venom/g body weight established 100% mortality at 10.0 μg/g or greater and 100% survival at 1.0 μg/g after 24 h.

Incapacitation and toxicity data are presented in Figure 5C–D. Juvenile venom is approx. 2× more effective in immobilizing lizard prey and approx. 2.5× more toxic to lizards than is adult venom.

Autopies at 24 h revealed that the lungs were the most extensively damaged tissue at all dose levels, often appearing dark red to black, with little remaining structural integrity. At the lowest doses (10.0 μg/g), much blood appeared in the throat, apparently from the lungs. At higher doses, little blood was present in the buccal cavity and was typically absent at the highest dose levels used (70 and 90 μg/g). Liver tissue appeared flaccid and greatly discolored, usually black-brown rather than the normal tan color. It was not possible to distinguish potential autolytic tissue changes from those induced by venom.

Discussion

Venom characteristics.—Rattlesnake venoms contain a variety of enzymatic components, many of which are lytic or degradative and could play a role in prey digestion. Proteases are perhaps the most important class of these enzymes, because the general lysis of structural proteins will enhance the exposure of the viscera of prey to stomach enzymes of the snake. Proteolytic enzymes are very common in crotaline venoms

(Tu et al., 1966; Kocholaty et al., 1971; Tu, 1982; Markland, 1983), and several proteases have been isolated from a single venom (e.g., *C. atrox*: Bjarnason and Tu, 1978; *C. ruber*: Mackessy, 1985). Together these proteases constitute a significant fraction of the whole venom. In the present study, large differences in protease activity levels were noted between adult and juvenile rattlesnake venoms (Fig. 2), suggesting that size-related increases in activity are related to other changes in the rattlesnakes' life histories.

Two other enzyme activities showed size/age related variation. L-amino acid oxidase activity tended to increase with age (Fig. 3), but the magnitude of change is not as great as that noted in *C. horridus horridus* (Bonilla et al., 1973). The enzyme also showed a great deal of substrate-specific variation in activity, and the above-noted differences may simply be a reflection of the different substrates and assays used. L-amino acid oxidase activity was detected in all venoms sampled, including the "white" venoms produced by some juvenile Pacific rattlesnakes. No functional role, other than the oxidative deamination of amino acids (a catabolic role) has been experimentally demonstrated for this ubiquitous venom component, but a role in protease activation has been suggested (Zeller, quoted in Russell et al., 1963:230). If L-amino acid oxidase does potentiate protease activity, then the concurrent increase in oxidase activity may complement increasing protease activity.

Phospholipase A_2 activity showed a significant inverse relation to size (Table 1). This enzyme is present in a wide variety of venoms and tissue types and has received much attention due to its usefulness as a membrane probe and, with some phospholipases A_2, as a neurotoxin. Several of the presynaptic neurotoxins of snake venoms have inherent phospholipase A_2 activity (Hawgood and Smith, 1977; Gopalakrishnakone et al., 1980; Lee et al., 1982). Greatly elevated levels of this enzyme were noted in the venoms of juvenile *C. v. helleri* and *C. v. oreganus* and may be responsible in part for the higher toxicity of juvenile venom toward lizard prey. Exonuclease and elastinolytic activities were assayed but did not show size-related variation, suggesting that their roles in venom function remain relatively constant. Hyaluronidase, the so-called "spreading factor," thought to be widely distributed in venoms (Iwanaga and Suzuki, 1979), was not detected in either juvenile or adult venom.

Biological role of venom.—Venom protease activity allows rattlesnakes to capitalize more effectively upon large mammalian prey and may facilitate utilization of a seasonally abundant food source, such as rodents. Although other large snakes could also dispatch such prey, protease activity of rattlesnake venom allows at least partial uncoupling of the dependence of digestion on ambient temperatures. Rattlesnakes may ingest prey of large volume without it decomposing due to slowed digestion at low temperatures (Thomas and Pough, 1979). In addition, by accelerating digestion, proteolytic venoms may allow rattlesnakes to expand foraging activities. This would explain the otherwise anomalous observation that the diminutive species *C. c. laterorepens* (Colorado desert sidewinder) produces venom with high protease activity (Table 2). This small desert snake is frequently encountered during conditions unfavorable for most snakes, i.e., cold (14 C) and windy nights; sidewinders are also active diurnally, when temperatures may approach 38 C (pers. obs; for detailed temperature/activity data see Brattstrom, 1965; Brown, 1970). Snake digestion shuts down or slows greatly under such conditions, and the digestive action of a highly proteolytic venom may allow sidewinders to feed at both high and low temperatures, when competition from other snakes would be minimal. There may in fact be no premium for the production of a degradative venom at "normal operating temperatures": the data of Thomas and Pough (1979) at 25 C suggest this, because no difference in degradation was noted between control and envenomated mice. Proteolytic activity in venoms may therefore represent an adaptation promoting efficient digestion in a variable environment.

Concomitant with an increase in protease activity is a decrease in venom toxicity. Venom from juvenile *C. v. helleri* and *C. v. oreganus* is much more toxic to *S. graciosus* than is adult venom (Fig. 5); a similar relation between toxicity to Swiss mice and age of snake exists for *C. v. viridis* (Fiero et al., 1972), *C. h. horridus* (Bonilla et al., 1973) and *C. v. oreganus* (Kardong, 1986). Venom of juvenile Pacific rattlesnakes also contains a higher percentage of, or a more active, phospholipase A_2 (Table 1).

Phospholipase A_2 is a common component of many snake venoms (Lee, 1979). This enzyme has been considered a major venom constituent contributing to the lethality of venom of *Bothrops alternatus* (Nisenbom et al., 1986). The

most toxic rattlesnake venoms, from *C. durissus terrificus* and *C. s. scutulatus*, both contain a two subunit neurotoxin, crotoxin (Brazil, 1966) and Mojave toxin (Bieber and Tu, 1974), respectively; phospholipase A_2 activity is associated with the basic B subunit of each (Breithaupt et al., 1971; Cate and Bieber, 1978) and is the major toxic factor in these venoms. More recently, a homologous toxin has been isolated from the venom of the midget faded rattlesnake, *C. v. concolor* (Aird and Kaiser, 1985). The increased toxicity of juvenile *C. v. helleri* and *C. v. oreganus* venom may result from higher phospholipase A_2 activity in these venoms, perhaps imparting a neurotoxic action analogous to that of crotoxin and Mojave toxin (Rael et al., 1986). An interesting corollary observation is that protease activity of *C. s. scutulatus* is quite low in adult snakes (Table 2), suggesting a possible condition of venom neoteny in this highly toxic species.

Ontogenetic changes occur in diet as well as venom composition. Newborn and young Pacific rattlesnakes are limited by size to a restricted selection of suitable prey: lizards, newborn rodents, and probably insects. Figure 4 illustrates the relative importance of lizard and mammal prey at a given size class. Although birds and lizards are occasionally taken by larger rattlesnakes at low frequency (Fitch and Twining, 1946; Fitch, 1949; Klauber, 1956), lizards make up a major portion of a young rattlesnake's diet (*C. v. helleri* and *C. v. oreganus*). Above 500 mm in length, snakes apparently feed on mammals exclusively. Several European species of *Vipera* show a similar ontogenetic shift in prey from lizards to small mammals (Saint-Girons, 1980). However, these data may not indicate prey preference as much as they suggest the availability of prey of suitable size for young rattlesnakes. *Uta stansburiana* and species of *Sceloporus* are common throughout most of the southern California range of *C. viridis* (Stebbins, 1966), and young rattlesnakes can dispatch most size classes of these lizards. On the other hand, most rodents, except newborn, are too large to be swallowed by rattlesnakes less than about 400 mm in length. For young Pacific rattlesnakes, lizards are the most commonly encountered, manageable prey, and in regions where lizards are uncommon, neonate mortality may be very high (Fitch and Twining, 1946).

The high toxicity and low protease activity of juvenile Pacific rattlesnake venoms represent an optimization of venom composition toward a specific prey type. Size constrains juvenile snakes to feed upon small prey which are readily digested by stomach enzymes, and high proteolytic activity is therefore "not needed." However, juvenile rattlesnakes, with a restricted potential prey repertoire and an inability to withstand protracted fasting (see hibernation weight loss data of Klauber, 1956 and Hirth, 1966), are operating under strong selective pressures favoring retention of suitable prey once it is located.

Rattlesnakes commonly employ a rapid strike and release pattern for the envenomation of prey, followed by relocation of prey via visual and olfactory cues (Klauber, 1956; Radcliffe et al., 1980; Kardong, 1986). This response represents a generally satisfactory compromise between avoiding retaliation of prey and losing prey altogether. However, this pattern is most typical of adult rattlesnakes feeding on active prey such as adult rodents. In this lab, juvenile Pacific rattlesnakes would most often strike and hold lizards (*U. stansburiana*) and newborn mice (pers. obs.). High toxicity of juvenile venom coupled with a strike-and-hold feeding response ensures that prey will be retained and prey struggle will be minimized.

As snakes grow, they begin to feed on larger mammalian prey which is bulky, and, due to a greatly lessened surface to volume ratio, is harder to digest (Pough and Groves, 1983). Rapid immobilization of prey is still a necessity to prevent envenomated prey from escaping beyond a relocatable distance. However, highly toxic venom may not be suitable for such prey, because their rapid death would preclude venom distribution throughout the body, and thus eliminate the digestive advantage afforded by high protease activity. Immobilizing prey via venom-induced shock allows proteolytic enzymes to be delocalized by the envenomated animal's circulatory system. The ontogenetic increase in protease activity compensates for the concurrent increase in "digestive resistance" of larger mammal prey by increasing the speed of visceral cavity rupture, in turn increasing the effective surface area available to stomach enzymes.

Rattlesnake venom has two main biological roles during feeding: immobilization of prey and initiation of digestion. Venoms are not a random collection of chemicals but are rather a highly specialized secretion which changes composition through development in a directed fashion. In the Pacific rattlesnakes *C. v. helleri* and *C. v. oreganus*, adult and juvenile snakes are

feeding on different types of prey, and venom composition varies ontogenetically in a manner consistent with maximizing venom effects toward specific prey at a specific point in time. Particular enzyme systems, specifically the increase of protease activity with size, offset environmental constraints and digestive problems associated with changes in diet. Venom ontogeny in these rattlesnakes follows a predictable pattern of development which may be expected in other species of large North American rattlesnakes as well.

Material examined.—San Diego Society of Natural History Museum (SDSNH) catalog numbers of specimens of *C. v. helleri* used for gut content analyses: 1724, 1733, 1995, 2180, 2932, 3778, 4096, 4305, 4307, 4334, 4383, 4570, 4728, 4825, 5163, 5165*, 5275, 7533, 7534, 7584, 7586, 7587, 7851, 7852, 8118, 8120, 8510, 8702, 9263, 9503, 10537, 10914, 16533, 16547, 18848, 20492, 22146, 22237, 22256, 22333, 22373, 22375, 22376, 22485, 22695, 22696, 22744, 23201, 24044, 24072, 24073, 25318, 25319, 26077, 26446, 26714, 26722, 26924, 26928, 26932, 27139, 27321, 27522*, 27536, 27722, 28013, 28480, 28555, 28556, 28639, 29014, 29120, 29276, 29568, 31320, 31323, 31411, 31762, 31763, 32104, 32305, 32670, 32967, 33252, 33405, 33930, 34080, 35100, 35312, 35314, 35387, 35670, 36179, 36212, 36252, 36337, 37399, 37423, 37472, 38302, 39603, 39713*, 39845, 39846, 40107, 40634, 41265, 41520, 41750, 42201, 42298, 42380, 42381, 42794*, 42881, 43098, 43176, 43354, 43356, 44077, 44078, 44086, 45125, 48058, 58235, 48236, 58237, 58240, 58243, 58245, 58424, 58440, 60204. *Contained lizard scales and mammal hairs.

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