

Rate and quantity of delivery of venom from honeybee stings

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To determine the rate and completeness of delivery of venom from honeybee stings, European bees were collected at the entrance of a hive and studied with the use of two laboratory models. In one model bees were induced to sting the shaved skin of anesthetized rabbits. The stings were removed from the skin at various time intervals after autotomization, and residual venom was assayed with a hemolytic method. In the other model the bees were induced to sting preweighed filter paper disks, which were weighed again after removal of the sting at various intervals. Results of both experiments were in agreement, showing that at least 90% of the venom sac contents were delivered within 20 seconds and that venom delivery was complete within 1 minute. The data suggest that a bee sting must be removed within a few seconds after autotomization to prevent anaphylaxis in an allergic person. The extensive variation found in the amount of venom delivered at each time point may explain inconsistencies in relationships among reactions to field stings, sting challenge testing, venom skin tests and RAST. (J ALLERGY CLIN IMMUNOL 1994;93:831-5.)

Key words: Venom delivery, kinetics, honeybee, *Apis mellifera*, sting, anaphylaxis

Envenomation by a honeybee is initiated by the insertion into the victim's skin of the stinging apparatus or sting. The shaft of the sting is comprised of two barbed stylets that slide back and forth on the underside of another barbed stylet, forming a channel through which venom passes from the venom sac in the base of the sting. Reciprocal axial movements of the stylets serve to progressively bury the sting in the skin and initiate flow of venom.^{1, 2} These movements are effected by rhythmic action of the motor apparatus in the base of the sting. This occurs as soon as the apparatus is denervated by separation of the sting from the abdomen when the bee pulls away from the embedded sting, a process known as autotomization (Fig. 1).

Quantitative information on venom delivery during the course of a bee sting may be helpful in the diagnosis and management of bee venom anaphylaxis. Knowledge of the completeness of venom delivery may help to relate the results of

sting challenge tests in the clinic or the responses of patients to stings in the field to the maximum doses of venom given in immunotherapy to those patients. Although our previous studies have shown that the average content of bee venom sacs is higher than the usual doses of venom given in immunotherapy,³ there is no published information on the fraction of the venom content of sacs that is delivered during the course of a single sting. In the case of multiple stings, which is now a possibility in warm climate regions of the United States since the arrival of Africanized bees, the total dose of venom delivered in a stinging attack can be calculated if the kinetics of venom delivery are known. The rate of delivery of venom from venom sacs would also help to determine how rapidly the sting apparatus needs to be removed from the skin after a sting occurs. This report provides the first description of the kinetics of venom delivery from *Apis mellifera*.

METHODS

European honeybees were collected near a hive entrance with an insect net and transported immediately to the laboratory in a cooler with ice. They were then tranquilized with CO₂ and transferred to holding containers where they were provided with both fresh water and a saturated sucrose solution if they were not used for sting studies immediately. They were kept away from drafts and in the dark at 23° C.

Rabbits were anesthetized with a mixture of ketamine (25 mg/kg), xylazine (8 mg/kg), and acepromazine

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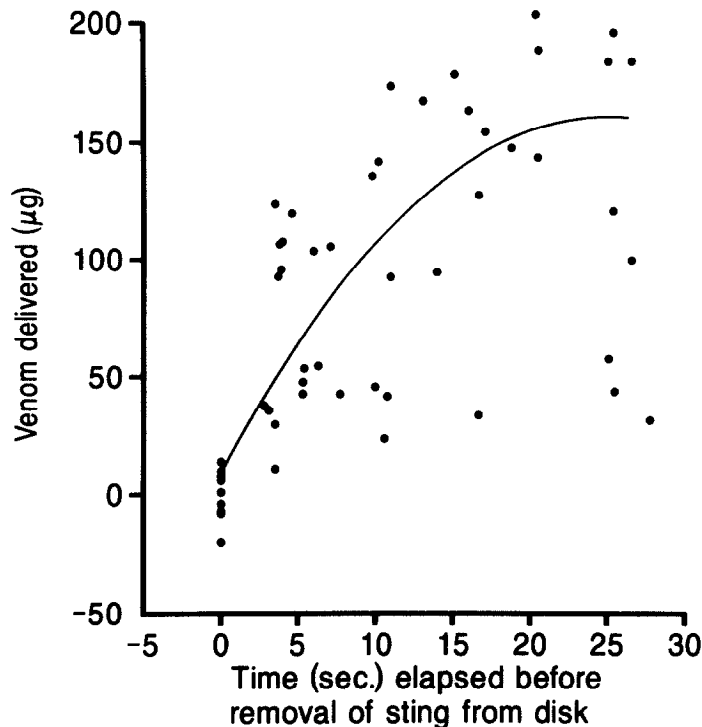


FIG. 3. Dry weight of venom delivered into filter paper disks per unit of time.

cules, Calif.). The disks were dried at 37° C for 4 hours, and each dry disk was kept in a stoppered plastic tube until it was weighed on a Cahn microbalance (Cahn Instruments, Inc., Cerritos, Calif.). Bees were induced to sting the disks, which were placed in aluminum foil boats immediately after autotomization. At various times after autotomization, the stings were grasped with forceps close to the paper surface and removed from the paper. The disks in the aluminum foil boats were dried again at 37° C and reweighed. The weight gains of the dried disks were corrected by subtraction of the average weight gain of control filter paper disks that were not stung.

RESULTS

In both rabbit skin and various artificial media, the autotomized sting was noted to embed itself progressively deeper over a period of approximately 30 seconds. By the end of this period, it was noted that at least two thirds of the length of the sting was embedded.

In the rabbit skin model venom in 63 sacs was depleted by 90% over a period of 20 seconds. There was a statistically significant negative relationship between residual venom and time ($p < 0.05$). No residual melittin could be detected at 40 seconds, indicating that the venom sac was empty (Fig. 2). To determine whether some melittin might have been lost in the handling of the venom sacs before the assay, stings (including their venom sacs) were removed from live bees,

chilled immediately, and processed identically to stings removed from rabbit skin. Venom sacs from the stings that were removed directly from bees were estimated to contain 82.5 µg of venom, approximately 60% of the average venom content of bee venom sacs found in a previous study of dead, frozen bees.⁴ This indicated that some venom was probably lost during manipulation of the stings.

In the paper stinging model the rate of increase in dry weight of venom delivered into the paper medium was similar to the rate of depletion of venom from the stings implanted in rabbit skin (Fig. 3). The relationship between venom delivery to the disk and time, analyzed by linear regression, was significant ($p < 0.05$). Although venom delivery varied considerably, the average weight gain of the disks at 20 seconds after autotomization was 140 µg, similar to the average venom sac contents of domestic honeybees. There was no tendency for further weight gain after this time, indicating that venom delivery was rapid and complete in less than 30 seconds.

DISCUSSION

Results of both methods used to study the kinetics of venom delivery from honeybee stings were similar in that the rate of depletion of residual venom from the sacs of stings implanted in rabbit skin was comparable to the rate of

TABLE I. Analysis of data shown in Fig. 3, showing distribution of amounts of venom delivered to paper disks at various elapsed times

Elapsed time (sec)	N	Percent of observations in the range (μg)					Median (μg)	Mean $\pm$ SD
		< 25	25-50	50-100	100-150	> 150		
2-7	17	5.9	35.3	23.5	35.3	0	53	71.5 $\pm$ 36.7
8-16	12	8.3	16.7	16.7	25.0	33.0	133	114.8 $\pm$ 57.6
> 16	14	0	21.4	7.1	28.6	42.9	148	141.9 $\pm$ 89.2

delivery of venom from stings implanted in absorbent paper. Even if the loss of venom from stings removed from rabbit skin was as high as suggested by a control experiment (40%), results of the cutaneous model are still consistent with very rapid venom delivery. Both methods also indicated that the sting reservoirs usually empty completely after autotomization. Comparability of results of *in vivo* and *in vitro* methods suggest that the kinetics of venom delivery into human skin are likely to be similar.

Delivery of venom from bee stings is much faster than has been assumed by those who recommend careful removal of the stinging apparatus embedded in the skin of an allergic person. Because substantial amounts of venom are delivered in the first 5 to 10 seconds, it may be necessary to remove the sting within a few seconds of implantation to try to prevent anaphylaxis in a person susceptible to systemic anaphylaxis. The results suggest that if the sting is not removed within 30 seconds from the time of implantation and autotomization, compression of the venom reservoir during sting removal will not inject more venom into the skin. In the case of a person who has been stung many times by hundreds of aggressive bees, removal of the stings in the emergency room is a cosmetic procedure that could be delayed until the patient has been treated for the effects of venom toxicity.

Knowledge that the venom sac usually empties completely allows estimation of total venom delivery from published data on bee venom sac contents.^{3, 4} This information is relevant to persons who have been stung many times by Africanized bees and provides the basis for calculation of potentially lethal doses of bee venom for human beings from animal median lethal doses. Assuming a median lethal dose of 3.5 mg/kg and an average venom delivery of 140 μg per sting,⁵ one could speculate that a 70 kg human being would have a 50% risk of death from 1750 stings. Al-

though this is consistent with case reports of deaths caused by Africanized bee stinging attacks, extrapolation of median lethal doses of toxins from small animals to human beings is fraught with uncertainty.

The variation among bees in the quantity of venom delivered into paper disks was great, reflecting a similar extent of variation of venom sac contents in individual bees, as observed previously.³ This finding is consistent with the complete emptying, after autotomization, of stings that varied in venom content. If the actual variation in the delivery of venom from stings in human skin approaches the variation found, this could explain the poor correlation that is often found between the effect of a single sting challenge and other measures of anaphylactic sensitivity such as venom skin tests, RAST, and field stings.⁶⁻¹¹ The severity of an allergic reaction to a single sting in the field may well depend on the venom content of the bee in question.

As shown in Table I, the median venom delivery into paper disks of 148 μg after 16 seconds is comparable with the average venom sac contents shown in our previous studies.³⁻⁵ This amount is three times the venom delivery previously reported.¹² Unfortunately, comparison of the data is impossible because of incomplete description of the methods in that study. Although 148 μg is two to three times the usually recommended dose for venom immunotherapy, comparison of therapeutic venom doses with venom sac contents may not be relevant to efficacy of immunotherapy given over a prolonged period. What may be more important is the previous expectation that tolerance of 50 μg venom doses by injection implied that a field sting would be tolerated relatively early in the course of immunotherapy. In the light of the present data, this now seems unlikely, and it might be prudent to increase bee venom doses in geographic regions where multiple bee stings are becoming a possibility.

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