

IMMUNIZATION IN WASP-STING ALLERGY THROUGH VENOM-REPOSITORIES AND PERIODIC INSECT STINGS^{1, 2}

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We have reported (1) that the patient with extreme allergy toward wasp stings can be protected for months by a one-visit, 2½-hr course that conveys 6 sacsful of wasp venom in about six divided doses by the endermal route. Although this probably represents the ultimate abbreviation of effective immunization with fluid venom, experience with emulsified allergen in hay fever (2) has suggested that the entire dose of venom might be tolerated in a single subcutaneous injection if incorporated in mineral oil. At the same time, it seemed likely that an already low incidence of adverse reactions to fluid venom might be further reduced, whereas the resulting immunity might be prolonged (3, 4). Accordingly, nine polistes-allergic persons were given trial repositories of polistes venom during 1960 and 1961, two other individuals with the same allergy being assigned to fluid venom to provide concurrent data for comparisons. Since most of the repository-treated group had taken prior courses of fluid venom, local and focal responses to the two forms could be compared in the same individual. The relative protective effects were uncovered by challenging the patient after each type of management with lively, stinging wasps (1) at various intervals after therapy, as stated in a preliminary report of findings (5). Less revealing of immunity were data procured through controlled testing of the skin and eye which were expected to reflect the acquisition of blocking antibodies as in earlier studies (1). Although post-treatment data were disappointing in this instance, conjunctival end points deter-

mined *before* therapy did serve a useful purpose, in that they substantiated the patient's history as far as the intensity of his sensitization was concerned, and thus threw light on his probable tolerance toward injected venom.

MATERIALS AND METHODS

Patients were accepted for the study only if they had suffered truly alarming reaction to polistes stings. Five of the group, for example, had lost or almost lost consciousness within 20 min of being stung. Hypotension must have been less marked in five others who described dizziness, profound emotional depression, exhaustion, or somnolence. Four of the group had vomited and three had exhibited profuse sweating. Whereas lingual edema had developed in three, only one recalled visual disturbances. There were generalized urticarial eruptions in six of the group, with pruritis that was most intense in soles, palms and scalp. Six reported dyspnea, two tachycardia, one violent "pounding in the head," and one noted a characteristic "foul taste."

All reported having received one or more prior innocuous wasp-stings, which presumably initiated sensitization. In addition, one clearly recalled that a week after his first adverse reaction generalized urticaria recurred, in a fashion that seems reminiscent of serum disease. Three of the group had taken courses of commercial, mixed hymenoptera (whole body) extract. In one instance, perennial injections of this type had been interrupted a year earlier because they had failed to prevent coma in the patient after a field encounter with polistes. Two others had so regularly experienced intense malaise after their year-round injections that they discontinued treatment 6 weeks and 25 days, respectively, before joining our experiment. No other members had been treated with any form of insect preparation.

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Antigen, in the form of aseptically extirpated venom sacs, was procured from lightly anesthetized, live polistes worker wasps that had been pinned onto a sterile cork, and swabbed with a 0.1% aqueous solution of benzalkonium. When venom was to be used for future tests or for multi-injection therapy in fluid form, the sacs were promptly covered with a small amount of sterile glycerin to preserve their potency and sterility during storage at 4 to 6°C through the ensuing winter and spring. Venom destined for emulsions was always obtained from live wasps on the day of treatment to circumvent any need for glycerin, which accelerates the release of antigen from emulsions (3).

Serial dilutions of venom were carried out on the day of tests or of therapy. After removal of the rubber cap from the 8- x 45-mm tube containing the sacs, venom was released by trituration with a carefully rounded glass rod. After the rod had been rinsed with a solution comprised of 0.036% KH_2PO_4 , 0.14% Na_2HPO_4 , .12 H_2O , and 0.5% NaCl , more of this diluent was added to give the desired concentration of venom, usually 25 sacsful/ml for testing or for endermal treatments, and 12 sacs/ml for emulsification purposes. The released venom was then freed from its fragmented sac walls by brief centrifugation and decantation.

To locate the minimal reactive doses (MRD) by endermal and by conjunctival instillation techniques (1, 3), a series of 12 doubled dilutions was set up. Whereas the 8 highest dilutions were employed for skin tests in a volume of 0.01 ml, the tube containing 0.09 sacs/ml (more conveniently expressed as 0.009 sacs/0.1 ml) served to initiate eye tests. Such instillations involved exactly 0.02 ml and were repeated at 5-min intervals with increasing strengths until reddening of the caruncle indicated the end point (6).

Emulsion (enough for one treatment) was formulated with lintfree, sterile material in a Wasserman tube, as follows:

Arlacel A (specially prepared for human use by Atlas Chemical Industries, Inc. of Wilmington, Del., and tested for nontoxicity in animals by Davenport's group at the University of Michigan) . . .	0.15 ml
U.S.P. light mineral oil (Atreol #9 of Atlantic Refining Co., Newark, N. J.)	0.9 ml

(In practice, it is simpler to combine the oil and surfactant, using 1.05 ml of the "1:6 Arlat")

Freshly removed venom (10 sacsful released into 0.83 ml of diluent) . .

0.75 ml

After the foregoing mixture of three components had been labeled "Pol. Arlat 1:6:5 containing $\frac{1}{2}$ sac/0.1 ml of emulsion," emulsification was achieved by forcing the material vigorously up and down in a 2-ml tuberculin syringe to which a sawed-off, 15-gauge needle had been firmly attached. To achieve maximal shearing of the aqueous phase during this manual mixing, it was important to press the blunted needle firmly against the bottom of the tube on each down-thrust. After 6 min, all the emulsion was drawn into the syringe for tests and therapy, the blunt needle now being replaced aseptically with one of 26 gauge. Success of emulsification was determined according to the following criteria—all as yet arbitrary. First, a globule dropped into a tube of thioglycollate broth (for determining sterility) should prove difficult to fragment and disseminate by the most vigorous agitation of the tube. The material should also be of such consistency that it maintains a pyramidal shape when touched off onto a glass slide. Microscopically, the homogeneous-appearing profusion of water droplets should measure 0.8 to 1μ in diameter, larger ones being encountered only once in perhaps six high-dry fields.

Prior to therapy, a drop of the emulsion was rubbed gently into a scratch on the patient's forearm. A wheal-and-flare response within 20 min reflected an extraordinary degree of allergy, only one such instance arising among the nine members of our experimental group. The exceptional man exhibited conjunctival responses to remarkably low concentrations of venom, and proved unable to tolerate our standard repository dose of six venom sacs in a single injection.

Although emulsions were injected on the day of their preparation without awaiting the outcome of cultures, none gave any sign of contamination during the ensuing 21 days. The same negative result had been procured on repeated occasions when we subjected Arlacel A, mineral oil, or a mixture of the two to sterility test, regardless of whether they had been autoclaved, passed through an ultrafine fritted glass filter, or cultured in original state.

The date assigned for venom repositories rested importantly on the availability of live worker wasps. Since the season of abundance for polistes is June through October in the writer's general vicinity and since about 2 months are required for the full immune response to emulsified allergen (3), early April is probably ideal for emulsion injections. Being a month late with our first trials in 1960, it seemed indicated to give all but one of the polistes repository recipients a sac or two of supplementary venom in fluid form promptly after the major treatment. Although more than ample time for maximal response was allowed by completing the repository treatments of 1961 during February and March, there was no way to know in advance whether resistance would persist into October. The problem was circumvented by introducing periodic stingings into the therapeutic regimen, since they offered the possibility of maintaining immunity throughout the year.

Focal and localized responses to each injection were described in detail in the patient's record, along with frequent listings of pulse rate and blood pressure. Repository sites were palpated whenever the patient came into the office, to detect any development of fibrosis, abscess, cyst, or new growth.

Sting tests with live insects, usually polistes but occasionally yellow jackets, were always conducted, initially, in the vicinity of the emergency crew at The New York Hospital. Furthermore, it was customary to have an intravascular drip of 5% dextrose solution running slowly throughout the experiment in readiness for untoward developments. Once an individual had exhibited full tolerance, however, he underwent future stingings in a routine office environment at the hospital. On at least one occasion per patient, the test was commenced by applying a nonstinging, male wasp (placebo) to the forearm for a few minutes, the aim being to ascertain the effect on pulse rate and blood pressure. When a female worker wasp was then substituted, care was taken to place her at a safe distance from visible veins. Once her stinger had penetrated the skin, the insect was held lightly in the stinging position for 2 min by means of a thumb forceps. The resulting wheal-and-flare reaction was outlined in ink at 20 min for later transfer to tracing paper. If additional wasps were to be

applied, an interval of 20 min was allowed between stingings.

Intermittent sting therapy. Because it was simpler than either repository or six-injection immunization and particularly because it offered the possibility of perennial protection, sting therapy was recommended to all adult members of the polistes group once they had demonstrated full tolerance. Beginning with intervals of 2 to 21 weeks, the time between such treatments was gradually lengthened until focal signs were induced by a sting or until at least 4 months had elapsed. Ultimately, we hoped it would prove feasible to restrict management to a few planned stingings yearly.

FINDINGS

Preliminary and post-treatment tests. A crude correlation appeared to exist between the intensity of a patient's sensitization as judged by history and the minimal reactive dose (MRD) determined for his conjunctiva during the initial visit. Patients BA and HU, for example, who had been rendered convulsive and unconscious within 5 min by the sting of a polistes wasp, required only 0.058 and 0.117 sacs/0.1 ml, respectively, for their conjunctival responses. FO and OS with the slightly higher end points of 0.234 and 0.312 described mental foginess but no actual "black-out" following a sting. The degree of hypotension must have been somewhat less in four other members of the group (HI, RO, AT and AN) who became weak but remained fully cognizant of such typical developments as visual aberrations, profuse sweating, vomiting, dyspnea, joint pains and generalized urticaria. For each the MRD was found to be 0.312 sacs/0.1 ml. The symptoms were similar though somewhat less marked and less rapid in onset for two (GU and AF) whose minimal requirements were found at 0.625 and who had experienced only widespread urticaria, dizziness and slight dyspnea. The 11th member, DU, had a MRD of 0.937 and no apparent hypotension following a polistes sting, symptoms being limited to generalized itching with urticaria and intense lumbar arthralgia.

Thus, it was a rather consistent finding that the conjunctival requirement was lower as the patient's story of encounter had been more alarming. Furthermore, the two with the lowest MRD (BA and HU) exhibited the least tolerance to-

ward injected venom when therapy was instituted. Because it is less reproducible, the endermal end point determined concurrently with the ophthalmic MRD proved rather less reliable as a clue to the intensity of the patient's sensitization (1, 3). Group requirements ranged from 0.000,5 to 0.019, with the exception of BA who developed a wheal-and-flare response to as little as 0.000,03 sacs/0.1 ml.

When both types of test were repeated following immunization (4 to 6 weeks after fluid venom treatment, 23 to 39 weeks after emulsion), the conjunctival MRD was usually found elevated 2- or 3-fold, endermal results providing inconsistent evidence for the acquisition of immunity.

Adverse reactions to emulsified and to fluid venom were compared in eight members who had received emulsified venom in the present experiment and courses of fluid venom in the past. One of the group, BA, was so susceptible by history and by test that he required individualized dosage, but the remaining seven had taken standard courses of fluid venom on 19 occasions. As the table indicates, 14 (74%) of these annual treatments had evoked no adverse subjective or circulatory developments (blood pressure and pulse rate). Although the remaining five treatments provoked focal signs lasting 10 to 30 min, these were too mild to require medical intervention and could be listed as $\pm$. There was uniform tolerance of repositories given the same seven individuals in 1960-1961, despite the administration of the standard six-sac dose in a single injection.

Difficulties encountered in immunizing the eighth member, BA, with either form of venom are reflected in the table. During his initial visit, focal signs were precipitated by each of the first two injections of 0.25 sac, a starting dose of fluid venom tolerated by all other members except HU. The adverse signs were rather persistent, abating spontaneously only after 71 and 39 min. A third trial with this amount that day, however, caused no trouble. After taking 0.5 sac 20 min later, BA exhibited not only erythema of the face, thorax and palms, but reported paresthesia of the chin, followed by sudden weakness, nausea, sweating and pallor. A decline in blood pressure from an initial 152/82 to 90/60 then induced us to inject 0.25 ml of 1:1000 epinephrine HCl into the muscle, which soon reversed the

reaction. Inasmuch as he considered the whole incident trivial as compared to his six former episodes of unconsciousness in the field, it has been listed as being of $1\frac{1}{2}$ intensity, our maximal grade being 3. Eight days later, a starting dose of 0.28 sac in fluid form was chosen on the ground that some immunity ought to have resulted from his preceding injections. He promptly developed a similar, although much less alarming, syndrome of overdosage (without hypotension). After 2 hr of gradual improvement, therapy was reinstituted with doses that rose slowly from 0.002 to 0.125 sac. Although this series of 13 injections was fully tolerated, a final dose of 0.09 sac again induced borderline focal signs. The total dose conveyed during the two-visit course now amounted to 2.5 sacs. Three weeks later, a field encounter with a yellow jacket resulted in an adverse response that was limited to the skin and required no medical intervention.

Prior emulsification of the antigen simplified BA's management the following year, an initial dose of a whole sac being fully tolerated. However, a second injection (in 54-min) that contained only half as much venom, led to erythemas of the face, palms, neck and sclerae which persisted for nearly 4 hr. A week later, the same schedule caused only a borderline cutaneous response. At this point, BA's tolerance for plain venom was challenged by an endermal dose of 0.25 sac in fluid form. Flushing, cephalic throbbing sensations, widespread urticaria, and a 16-mm reduction in systolic blood pressure resulted in 10 min. Although $1\frac{1}{4}$ hr elapsed before complete spontaneous reversal occurred, another injection of emulsion was given at 1 hr. This final dose of 0.5 sac caused no exacerbation of signs. Full tolerance for emulsified venom was exhibited the following year, when a total of two sacs of polistes venom was given under the skin in five divided doses during a single visit in March, 1961. Polistes still being abundant 6 months later, it seemed indicated to give an aqueous booster stimulus in late August. It will be noted in the table that four long, multi-injection visits were employed to bring the total dose to our prescribed standard of six sacs. We had become sufficiently encouraged by an obvious increase in BA's tolerance for injected antigen, and by a slight upward shift detected on November 15th in the MRD of his skin (0.000,3) and

TABLE I

Date	Therapy (Polistes)			Challenges (Number of Insects)	Adverse Reaction		
	State of Venom	No. of Injections	Dose in Sacs		Degree	Started in:	Lasted for:
						<i>min</i>	<i>min</i>
AF 6-24-59	Plain	7	6.00		±	7	10
4-29-60	Emulsion	1	6.00		0		
4-29-60	Plain	3	1.00		0		
10-21-60				2 pol. ^{a, b}	0		
11-29-60				1 pol.	0		
2-3-61				1 pol.	0		
5-8-61	Emulsion	1	5.00		0		
AN 9-9-57	Plain (y.j.) ^a	5	6.50		±0	30	30
11-13-57				1 pol. (field)	0		
6-2-58	Plain	6	6.00		0		
8-15-58				2 h.bee ^a (field)	0		
5-5-59	Plain	6	6.00		±	120	3 da.
4-20-60	Emulsion	1	6.00		0		
7-5-60				1 h.bee (field)	0		
3-2-61	Emulsion	1	6.00		0		
AT 4-30-58	Plain	9	6.00		±	30	3½ hr.
5-4-59	Plain	6	6.00		±	90	3 hr.
6-12-59				1 pol. (field)	0		
4-19-60	Emulsion	2	5.00		0		
4-20-60	Plain	2	1.00		0		
9-30-60				1 pol. (field)	±	15	15
12-1-60				1 pol.	0		
1-10-61				1 pol.	0		
4-10-61				1 pol.	0		
8-1-61				1 pol. ^b	0		
11-1-61				1 pol.	0		
DU 6-21-56	Plain	6	5.40		0		
9-7-56				1 y.j.	0		
6-10-57	Plain	6	6.25		0		
6-11-58	Plain	6	6.0		0		
7-30-58				2 pol.; 1 y.j. ^b	0		
5-6-59	Plain	6	6.00		0		
3-1-60				1 pol. (field)	0		
4-20-60	Plain	6	6.00		0		
5-7-60				1 pol. (field)	0		
6-3-60				1 pol. (field)	0		
10-27-60				2 pol.; 1 y.j.	0		
11-23-61				1 pol.	0		
2-1-61				1 pol.	0		
5-2-61				1 pol.	0		
8-15-61				1 pol.	0		
9-4-61				2 y.j. (field)	0		
9-5-61				1 b.f. ^a (field)	0		
10-26-61				2 pol.	0		

TABLE I—Continued

Date	Therapy (Polistes)			Challenges (Number of Insects)	Adverse Reaction		
	State of Venom	No. of Injec- tions	Dose in Sacs		Degree	Started in:	Lasted for:
						<i>min</i>	<i>min</i>
FO 5-2-60	Emulsion	1	6.00		0		
5-2-60	Plain	3	1.50		± 0	27	10
10-26-60				2 pol.	0		
11-17-60				1 pol.	0		
1-11-61				1 pol.	0		
3-6-61				1 pol.	0		
5-15-61				2 pol.	0		
8-8-61				1 pol.	0		
10-25-61				1 pol. (queen)	+	7	65
1-2-62				1 pol.	± 0	5	19
GU 5-25-55	Plain	6	6.00		0		
6-8-55				2 pol.	$\pm$	24 hr.	16 hr.
6-21-56	Plain	6	8.00		0		
9-13-56				2 pol.	0		
6-10-57	Plain	6	6.25		0		
9-25-57				1 pol. ^b	0		
5-26-58	Plain	7	6.00		0		
5-6-59	Plain	6	6.00		0		
4-29-60	Emulsion	1	6.00		0		
4-29-60	Plain	3	1.00		0		
11-9-60				2 pol.	0		
11-23-60				1 pol.	0		
1-18-61				1 pol.	0		
3-29-61				1 pol.	0		
6-26-61				1 pol.	0		
10-11-61				1 pol.	0		
11-28-61				1 pol.	0		
HI 6-12-57	Plain (y.j.)	5	6.00		0		
6-12-57	Plain	5	6.25		$\pm$	25	30
6-6-58	Plain (y.j.)	7	6.25		0		
6-6-58	Plain	1	3.00		0		
8-19-58				1 y.j.; ^b 1 pol.	0		
5-6-59	Plain	6	6.75		0		
8-8-59				1 pol. (field)	0		
5-2-60	Emulsion	1	7.00		0		
5-2-60	Plain	3	2.00		0		
11-1-60				2 pol. ^b	0		
12-11-60				2 pol.	0		
2-18-61				1 pol.	0		
5-24-61				1 pol.	0		
10-10-61				1 pol.	0		
OS 5-28-58	Plain	6	6.00		0		
8-5-58				3 pol. ^b	0		
5-5-59	Plain	6	6.00		0		

TABLE I—Continued

Date	Therapy (Polistes)			Challenges	Adverse Reaction		
	State of Venom	No. of Injections	Dose in Sacs	(Number of Insects)	Degree	Started in:	Lasted for:
						<i>min</i>	<i>min</i>
OS 4-20-60	Plain	6	6.00		0		
2-10-61	Emulsion	1	6.00		0		
9-6-61				1 pol.	±	32	1
11-9-61				1 pol.	±	2	16
1-10-62				1 pol.	±0	15	9
RO 6-2-58	Plain	6	5.50		0		
7-28-58				3 pol. ^b	0		
5-5-59	Plain	5	6.00		0		
8-2-59				1 pol.	0		
4-26-60	Emulsion	1	6.00		0		
4-26-60	Plain	3	1.00		0		
10-20-60				3 pol.	0		
11-16-60				1 pol.	0		
1-11-61				1 pol.	0		
3-7-61				1 pol.	0		
7-14-61				1 pol. ^b	0		
11-22-61				1 pol.	±0	4	21
BA 6-24-59	Plain	1	0.25		±	14	71
	Plain	1	0.25		±	12	39
	Plain	1	0.25		0		
	Plain	1	0.50		+½	17	90
7-2-59	Plain	1	0.28		+	4	105
	Plain	13	0.88		0		
	Plain	1	0.09		±	17	30
7-23-59				1 y.j. (field)	±	30	3 da.
					+	120	45
4-20-60	Emulsion	1	1.00		0		
	Emulsion	1	0.50		+	14	225
4-27-60	Emulsion	1	1.00		0		
	Emulsion	1	0.50		±	33	101
	Plain	1	0.25		+	10	77
	Emulsion	1	0.50		0		
3-2-61	Emulsion	1	1.00		0		
	Emulsion	1	0.25		0		
	Emulsion	3	0.75		0		
8-24-61	Plain	1	0.13		±	12	7 hr.
	Plain	1	0.13		0		
	Plain	3	0.38		0		
10-4-61	Plain	1	0.10		0		
	Plain	1	0.10		0		
	Plain	1	0.15		±	21	74
	Plain	1	0.10		0		
10-11-61	Plain	1	0.09		0		
	Plain	3	0.28		0		
	Plain	1	0.09		±	14	79
	Plain	4	0.50		0		
	Plain	2	0.50		0		
	Plain	1	0.25		±	3	3 hr.
					+½	3 hr.	½ hr.

TABLE I—Continued

Date	Therapy (Polistes)			Challenges	Adverse Reaction		
	State of Venom	No. of Injections	Dose in Sacs	(Number of Insects)	Degree	Started in: <i>min</i>	Lasted for: <i>min</i>
BA 10-25-61	Plain	1	0.19		0		
	Plain	1	0.13		±	11	2 hr.
	Plain	1	0.13		0		
	Plain	1	0.25		0		
	Plain	5	1.25		0		
11-2-61	Plain	1	0.25		±0	20	3 hr.
	Plain	7	1.63		0		
11-15-61				2 pol.	0		
11-29-61				2 pol.	±0	22½	41
HU 6-14-61	Plain	1	0.25		++	9	20
	Plain	1	0.001		0		
	Plain	1	0.02		0		
	Plain	11	0.31		0		
	Plain	1	0.25		±	12	17
	Plain	3	0.48		0		
	Plain	1	0.25		±	10	45
	Plain	2	0.50		0		
8-7-61				1 y.j. (field)	0		
9-14-61				1 pol. ^b	++	7	75

^a Pol. = polistes; h.bee = honey bee; y.j. = yellow jacket; b.f. = baldfaced hornet.

^b Also a preliminary test with a male wasp (placebo).

conjunctiva (0.156) that we exposed him to stings by two polistes worker wasps on that date. There being no adverse sequelae, the feat was repeated, with almost equal fortune, 2 weeks later.

The three remaining members of the group (DU, HU and FO) received venom in one form only. The first is a physician who exhibited full tolerance toward five annual treatments comprised of 6 sacsful of venom given in six injections each. He has been on intermittent sting therapy for the past 1½ years. HU appears to pose the same problem as BA, for he was quickly reduced to borderline unconsciousness by the first standard dose of fluid venom. By resorting to minute subsequent amounts, we succeeded during the next 5 hr in achieving a total dose for the day of 2 sacs, despite two minor focal responses (see Table I). This apparently protected him against an accidental sting by a yellow jacket at 8 weeks but not against a captive polistes 5½ weeks later. The last patient, FO, took his primary injection in the form of a single, 6-sac repository. Because of the imminence of the polistes season,

he was immediately given three supplementary endermal treatments containing 1½ sacs in fluid form. A borderline focal reaction promptly resulted. In 25 weeks, when the fluid venom's influence had probably ended, he was deliberately stung by two polistes workers without incident. His responses to periodic stings during the next 14 months will be discussed under that heading.

Localized reactions to therapeutic venom will be mentioned only briefly because the recollection of most patients for moderate developments is unreliable. Accurate accounts are available, however, for some of the marked reactions. OS, for example, noted local erythema, induration, and slight swelling over an area 8 in. wide for a week after receiving our standard series of endermal injections of fluid venom in her arm. The treatment, incidentally, left her exhausted for 14 days. When the course was repeated the following year, such after-effects were "less violent." On the other hand, emulsified venom given in 1961 evoked a localized reaction that was only 1½ in. wide and disappeared on the

4th day, with very little associated fatigue. GU likewise volunteered the information that his localized responses, as well as an accompanying sense of *ennui*, were decidedly less after emulsified than after fluid venom. Both patients experienced still less discomfort when subjected to planned insect stings. Nevertheless, the patient AF felt so listless for a week after receiving venom in any form that she forsook sting therapy after three treatments and returned to once-yearly repository management, thereby reversing the choice of most members.

Nodules at times persisted beyond the presumed 6-month period of effectiveness of the repository. Whereas AT, AF and GU gave no palpable evidence of nodule formation at 40 days, 24 weeks, and $6\frac{1}{2}$ months, respectively, OS still exhibited a smooth, soft, elastic disk $1\frac{1}{2} \times 2$ cm wide when her subcutaneous injection site was last examined at $6\frac{1}{4}$ months. BA's fibrotic reactions to his first repositories measured 2 and 5 mm a year later, whereas those following his 1961 treatments with emulsion were only 2 mm wide at 5 months and could not be detected at 10 months. Two other recipients had detectable residues about $1\frac{1}{2}$ years after repository treatment, the thin pliable disk measuring $1\frac{1}{4}$ cm in the case of RO, whereas it was 7 mm wide in FO. No palpation data are as yet available for AN and HI.

To date there have been no abscesses, cysts, or new growths at injection sites.

Abbreviation of treatment time was achieved through emulsification. For eight typical members (excluding HU and BA), the time required to complete a standard course of 5 to 7 sacsful of venom in fluid form ranged from $1\frac{1}{4}$ to $4\frac{1}{4}$ hr, averaging 2 hr. In contrast, a repository was placed under the skin in about a minute (AT preferred to take his first emulsion in two injections). Because of the pioneering nature of the repository method in wasp allergy, subjects were retained for 45 min of observation.

The protective value of therapy was judged by deliberate and accidental challenges with live insects. It was possible to apply this crucial test of effectiveness to both forms of venom in the instance of six of the experimental subjects who had received at least one standard course of fluid venom in past years and a repository in the present experiment, both types of therapy being followed by challenges. These members (AN, AT,

GU, HI, OS and RO) had been stung by one to three insects during a total of 11 encounters after fluid venom therapy. Of these stings, none of the four inflicted in the field and only one of the six occurring in the hospital evoked the slightest focal reaction, despite the passage of $5\frac{1}{2}$ to $13\frac{1}{2}$ weeks since therapy. The exception was GU, who complained of fatigue the day after he was stung by two polistes. Excellent clinical resistance was again apparent when four of the foregoing members were stung following emulsion therapy. AN, for example, experienced only local discomfort when a honeybee stung his foot at 11 weeks, whereas deliberate exposures to two or three polistes evoked no adverse responses in HI, GU, and RO, despite post-treatment intervals ranging to 25 weeks. Borderline reactions to challenge did arise, however, in two of the others given repositories. One was engendered in AT 23 weeks after therapy when a polistes landed on his scalp in a distinctly unfriendly spirit. Within 15 minutes "thumb-nail" sized hives and bright erythema covered both forearms but shortly yielded to an oral antihistamine. In the other instance (OS), 30 weeks had elapsed since treatment when a lively polistes applied to her forearm evoked a fleeting sense of fullness in lips and palms without visible change.

Thus, among the six members subjected to both forms of therapy, $\frac{10}{11}$ of the challenges given after fluid venom and $\frac{4}{6}$ of those given after emulsion therapy evoked no adverse developments, whereas the remaining three challenges caused trivial discomfort.

In addition to these results in the six dually-treated individuals, there were uneventful, deliberate challenges in two others, AF and FO, with two polistes each, 25 weeks after repository therapy. There was no corresponding information for fluid venom therapy.

The three remaining members of the group of 11 were stung after immunization with plain venom only. In one way or another, each patient was exceptional. DU, for example, was stung repeatedly in the field, not only by polistes but also by yellow jackets and even by bald-faced hornets, there being an episode after four of his five annual courses of fluid venom—all proving innocuous. In fact, he unwittingly introduced us to the idea of intermittent sting therapy by encountering polistes 2 weeks and again 5 weeks, after his 1960 one-visit series of six injections.

BA was set apart not only by his extreme susceptibility to polistes but by a curious response he gave to a field encounter with a yellow jacket 3 weeks after two turbulent therapeutic sessions in 1959. In addition to prompt and extensive inflammation about the sting on the forearm, he observed that all the areas that had been tested with yellow jacket venom a month earlier lit up within $\frac{1}{2}$ hour of the stinging and remained pruritic for 3 days. Two hours after the sting, generalized urticaria developed but abated spontaneously. BA had no encounters after emulsion treatments in 1960 and in 1961. In preparation for the several remaining months of the polistes season, and with a deliberate sting test in view, we gave him intensive and protracted booster stimulation between August 24 and November 2, 1961, achieving a total dose of 6 sacsful of fluid venom. Two weeks later, as the table shows, he tolerated fully not only one but two polistes' stings in the hospital. After another 2 weeks, the exposure was repeated with almost equal success, as an introduction to sting therapy. His only adverse response was a suggestive flushing of the face for 10 min.

The story of the last member, HU, is brief and alarming. Encouraged by the fact that he had exhibited no adverse effects after an accidental sting by a yellow jacket 2 months after a long, stormy, one-visit course that resulted in his receiving 2 sacs of polistes venom in fluid form, we applied a polistes worker wasp to his forearm for 2 sec 5 weeks later. An alarming generalized reaction commenced in 7 min with tingling of the hands, flushing of face, palms, and thorax, drenching sweats, repeated retching, and profound apprehension. Meantime, a baseline blood pressure reading of 150/88 steadily fell until it reached 72/50. At this point, 10 min after the sting, 0.5 mg of phenylephrine-HCl was injected into the intravascular drip tubing, the foot of the table was elevated, and an O₂ mask was intermittently applied to the face to discourage hypoxia. A second dose of the adrenergic amine was given 10 min after the first, and recovery began shortly. One-half hour after the sting, the patient became (and thereafter remained) normotensive. Within 2 hr he was ready to set out on his 5-hr trip home. There were no further difficulties.

Periodic stings as a form of perennial therapy. As stated previously, all patients who exhibited

tolerance for deliberate stings were arbitrarily placed on a regimen of intermittent exposures in the hope that immunity would thereby be maintained. With three exceptions (HU whose safe therapy has yet to be designed, AN who has not yet been invited to join the program because of his youth, and DU who initiated the scheme after his fifth annual course of fluid venom), all those on sting therapy had received recent repository therapy. In addition to the initial sting of each, which was arbitrarily regarded as a test rather than a treatment, there have been 41 deliberate exposures among the experimental group as a whole. Focal signs have ensued in only three members, OS, RO and FO, on a total of five occasions and were trivial with one exception. FO, after exhibiting full tolerance to his fifth sting treatment in August, 1961, developed erythema of palms, face and thorax, as well as transitory nausea, 7 min after a sting in October. His blood pressure dropped somewhat, but never below 110/70. No medical intervention was required beyond the 10-min application of a venous tourniquet above the sting site. Whealing at the latter achieved a width of $2\frac{1}{2}$ in. In view of the finding that the MRD of his eye was unchanged from 0.234 where it had been located a year earlier on a day that two polistes' stings had been fully tolerated, we attributed the recent focal reaction to the size of the insect employed. She was, as a matter of fact, a large queen. Furthermore, as becomes a queen, she was given unusual leeway in the performance of her task. Restrained by only one wing, she wheeled about and punctured the forearm seven times! Supporting this explanation of FO's reaction was the almost uneventful outcome of his next treatment; in January 1962 an average-sized insect caused no disturbance beyond a slight, short-lived flushing of the face.

COMMENTS

Although the foregoing data indicate that 6 sacsful of venom in fluid or in emulsified form afford protection against one to three insect stings for at least 4 to 6 months, the time limits of this immunity have yet to be delineated as to onset, height and duration. In other words, how many more than one to three insects would have to be applied to a patient's forearm to overstep his tolerance? And how much longer than $7\frac{1}{2}$ months after repository therapy could OS be stung by a polistes without unequivocal adverse

developments? Whereas most of the emulsion-treated members were challenged some 6 months post-therapy, would they have exhibited similar tolerance 4 weeks after treatment, or perhaps 7 months later? Since the answers to such questions necessitate systematic testing over an increasing span of time until focal manifestations appear after a specified number of stings, one might question from the practical viewpoint whether those answers are worth the possible cost in terms of patient-safety and physician-stress. Perhaps we should rest content for the time being in the knowledge that a practical immunity has been achieved—practical in degree, because more than one to three insects are rarely implicated in an accidental encounter; practical in duration, because off-season field stings are a rarity. In any event, should unusual winter exposures appear likely, one can always insure perennial immunity by repeating therapy every 6 months; or, more simply by inflicting planned stings at, say, 4-month intervals.

It should be remarked at this point that venoms and whole body extracts of wasps are primary irritants capable of evoking a wheal-and-flare response in anyone. Hence, only those tests which locate the minimal reactive dose will separate allergic from normal subjects (1). In addition, it has become increasingly apparent that wasp-sting allergy is analogous to serum disease, an antigen-antibody disorder to which the general population, under proper conditions of parenteral exposure, is susceptible. For this reason, and especially because periarteritis and related diseases could conceivably eventuate, physicians should exercise great restraint in the use of insect preparations. Indeed, we are convinced that routine testing, even with the scratch method, should be avoided, the etiology of a field reaction being uncovered by history and by a knowledge of the circumstances rather than by the results of any type of test. Once established, the offender's venom should be used to determine the MRD so that the physician can use substandard amounts of venom to institute therapy in the unduly reactive patient. Only those individuals for whom a next exposure may prove disastrous should, however, be subjected to injection therapy; venom rather than whole body extracts should be the antigen, and the amount should be restricted to the least that suffices for protection. All other wasp-allergic

persons can be taught to avoid the usual haunts of the insects (7, 8) and, in the event of a critical reaction to field sting, to use sublingual isoproterenol HCl or intramuscular epinephrine HCl (1:2000 0.5 ml "Ampins" of Moore Kirk Laboratories, Inc., Worcester, Mass.).

We confess that these therapeutic restraints were not exercised at the outset when, in the middle 1940's, some means had to be devised to protect a physician's sting-allergic mother against a third episode of unconsciousness. After lyophilized bee-sting antigen, which we employed initially for experimental immunization against her honeybee and yellow jacket allergies, disappeared from the market, extracts of whole bodies were prepared from hives of live yellow jackets. For the first few years, large amounts of these preparations were employed in long, multi-visit courses to circumvent the risk of under-treatment. Smaller amounts were used in emulsified form in 1948 and 1949. Switching by 1953 to pure venom removed from live wasps, annual allotments of fluid venom were gradually lowered from 40 sacsful in four visits to 6 sacsful in one visit. Meantime, a sufficient number of planned and accidental stings among the members of our small but growing group assured us that the diminishing annual doses were still providing practical protection. More recent experience with over 50 such "anaphylactic" patients has indicated that, with rare exceptions, there is universal tolerance to what has become a standard dose of 6 sacsful of venom in fluid or in emulsified form. Either type of one-visit treatment confers satisfactory immunity against the planned or accidental stings of one to four wasps. Motivation to reduce this annual dose has been dampened by the ease and safety of giving the entire allotment in one injection in emulsified state.

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SUMMARY AND CONCLUSIONS

Of 11 persons with alarming immediate reactions to polistes stings, 9 have been immunized with Arlacel:petrolatum:venom emulsions during 1960 and 1961, a total of 12 such annual treat-

ments being given the group. For all but one member (BA), the repository was comprised of 1.2 ml containing all the venom of six live polistes workers, placed under the skin in a single injection (patient AT preferred a divided dose). There were no adverse effects either focally at the time of therapy or locally during the ensuing year or two. Seven of these members had been immunized, in one or more preceding years, with the same annual dose administered in fluid form during a one-visit, 2½-hr course that employed five to seven endermal injections. Five of their 19 treatments with fluid venom had, in contrast to the fully tolerated repositories, evoked borderline focal signs. In addition to these dually-treated patients, there was one physician who had tolerated five annual, 6-sac endermal courses of fluid venom, and another man who accepted uneventfully the full standard dose of venom in emulsified form.

Repositories built up a satisfactory degree of immunity as judged by six planned and two accidental stings by one to three polistes inflicted on eight members at intervals as great as 7½ months after treatment. Only two trivial focal reactions were engendered. Resistance appeared to be equally good after the standard series of fluid venom injections, for there was only one trivial adverse development among 16 challenges that were made as soon as 13 days or as late as 41½ weeks after therapy.

Emulsification decreased the venom's capacity to evoke local and focal responses. There were no cysts, abscesses, or new-growths at the repository sites.

In an effort to provide year-round protection, 8 of the 11 members have, since their exhibition of tolerance toward stinging wasps, been transferred 2 to 21 weeks later to a regimen of intermittent stinging. Five trivial and one mild focal response have been engendered by 41 such sting treatments. The latter serve not only as probable booster stimuli but also as periodic tests of immunity.

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