

Effect of bee venom on experimental arthritis*

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Stimulation of cells and tissues, whether by mechanical, hormonal, or neurological means, results in the increased biosynthesis of prostaglandins (Ramwell and Shaw, 1970). It has been suggested that one such stimulus is inflammation, in the course of which phospholipases are freed from the lysosomes of phagocytes. These enzymes can cleave phospholipids of the cell membrane to yield arachidonic acid, which is in turn converted to prostaglandins by freely available tissue enzymes (Anderson, Brocklehurst, and Willis, 1971). Phospholipases are also among the most active components of a number of animal venoms (Eliasson, 1959), such as bee venom and snake venom, which both contain phospholipase A and appear to release prostaglandins from tissues (Vogt, Meyer, Kunze, Luft, and Babilli, 1969). Since it has been observed that prostaglandin E_1 (PGE_1) prevents and suppresses adjuvant arthritis in rats (Zurier and Quagliata, 1971), it was considered possible that injections of venom in rats with adjuvant disease might have an effect similar to that of treatment with prostaglandins. Because of its venerable position in the medical and folk lore of Europe and Asia, the venom of the honey bee (*Apis mellifera*) was used. Bee sting or bee venom therapy has been reported to be effective in treatment of the rheumatic diseases (Maberly, 1910; Beck, 1935), and is widely used, even at the present time, in many regions of the U.S.S.R. (Zaitsev and Poriadin, 1964).

Adjuvant disease in the rat includes a severe and persistent polyarthritis, which appears 10 to 14 days after a single intradermal injection of complete Freund's adjuvant (Pearson and Wood, 1959) and is widely accepted as a useful animal model of human disease. The data obtained in these studies indicate that treatment of rats with whole bee venom from the time of adjuvant injection does in fact suppress adjuvant arthritis, whereas treatment with the known fractions of bee venom alone (including phospholipase A) does not reduce the inflammatory response. When treatment is delayed until arthritis appears, bee venom treatment does not alter the course of disease. Injections of whole bee venom result in rapid elevation of serum corticosterone concentration and bee venom

therapy does not prevent arthritis in adrenalectomized rats. The findings suggest that bee venom suppression of adjuvant arthritis is mediated, at least in part, through adrenal gland stimulation. These observations stand in contrast to the findings that PGE_1 suppresses adjuvant arthritis in adrenalectomized rats and therefore suggest that the effects of bee venom are not necessarily related to the release of prostaglandins.

Material and methods

Adjuvant disease was induced in male Carworth CFE rats (250 ± 25 g.) by intradermal injection into a hind paw of 300 μ g. killed *Mycobacterium tuberculosis* (Difco, Detroit) in 0.05 ml. mineral oil. Rats were housed in groups of four and allowed water and food (pellets of Purina Lab Chow) *ad libitum*. Adrenalectomized rats were given a 1.0 per cent. NaCl solution to drink, mineral/salt blocks were placed in their cages, and the rats were not used for experiments until 30 days after adrenalectomy. Two experimental groups were treated with either 300 or 500 μ g. whole bee venom subcutaneously twice daily for 21 consecutive days from time of adjuvant injection (Day 0). A control group was treated with saline. Other groups of rats were treated with either bee venom intraperitoneally, or melittin, apamin, or phospholipase A subcutaneously. The daily dose of melittin, apamin, and phospholipase A used corresponded to the amount of each fraction contained in 1 mg. whole bee venom. The severity of arthritis in the three uninjected paws was evaluated as described earlier (Quagliata, Sanders, and Gardner, 1969). A final group of rats was treated with whole bee venom (500 μ g. subcutaneously twice daily) beginning on Day 17, at which time polyarthritis was established.

Immune response to sheep red blood cells was determined as described by Levine and Levytska (1967).

Serum corticosterone levels were determined by Dr. Ralph Peterson, New York Hospital, using the method described by Peterson (1957).

Prostaglandin E_1 was a gift from Dr. John Pike, Upjohn Co., Kalamazoo; the dried bee venom was provided by Mr. Charles Mraz, Middlebury, Vermont. Melittin, apamin, and phospholipase A were supplied by Dr. William H. Shipman, Department of the Navy Undersea Research and Development Center, San Diego. The preparation of these fractions has been described by Cole and Shipman (1969).

Results

The effects on adjuvant arthritis of the subcutaneous administration of whole bee venom and of its known constituents are documented in Fig. 1. Whereas rats treated with bee venom from Days 0 to 20 developed little or no arthritis, those injected with melittin, phospholipase A, or apamin (the major constituents of whole bee venom) had no significant amelioration of their polyarthritis. There was no rebound or escape from protection by bee venom in those animals observed for 14 days after treatment was stopped (Fig. 1). In contrast to subcutaneous treatment, intraperitoneal injections of whole venom (at equivalent dosage) did not prevent disease (Fig. 2). The result of subcutaneous administration of a lesser dose (300 μ g. twice daily) of bee venom is also documented in Fig.

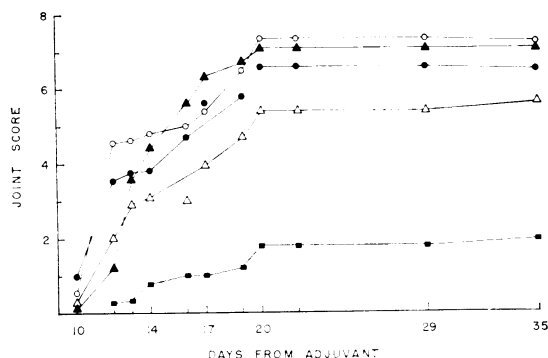


FIG. 1 Effect on adjuvant arthritis of whole bee venom and its constituents. Each point represents mean score for the three uninjected paws (12 rats per group). Treatment divided into two equal subcutaneous doses (Days 0-20). ■: Whole bee venom 1 mg. daily; △: Melittin 0.5 mg. daily; ●: Phospholipase A 0.15 mg. daily; ▲: Apamin 0.1 mg. daily; ○: saline-treated controls

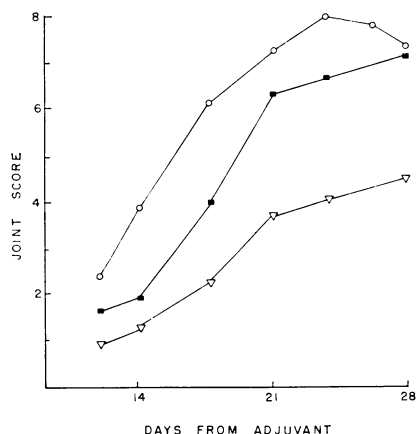


FIG. 2 Effect of bee venom on adjuvant arthritis. Each point represents mean arthritic score for the three uninjected paws (10 rats/group). ○: Control; ■: Bee venom intraperitoneally 375 μ g. twice daily (Days 0-20); ▽: Bee venom subcutaneously 300 μ g. twice daily (Days 0-20)

2. Although the average joint scores were significantly lower as compared to control rats ($P < 0.05$), results were uneven and three rats in this group of ten developed severe arthritis. Other than local pain associated with injections, we observed no adverse effects of treatment with bee venom or its fractions. Rats treated with 500 μ g. bee venom twice daily appeared more active, did not lose weight, and did not develop the leucocytosis and anaemia characteristic of the disease. These were, however, regularly documented in the untreated group and in those receiving injections of the fractions. Nor was the number of circulating lymphocytes diminished significantly by treatment with bee venom (Table I, overleaf).

When treatment with whole venom (500 μ g. twice daily) was begun on Day 17 (*i.e.* when moderate to severe polyarthritis had developed) the course of the disease was not significantly altered during a 15-day treatment period.

To assess the immunological competence of these animals, five rats of each group were injected intraperitoneally with a suspension (10 per cent., 1 ml.) of washed sheep red blood cells on Day 15. The brisk antibody response observed 7 days later in control (saline-treated) animals was only modestly reduced by treatment with whole bee venom and its fractions (Table II, overleaf).

The effects of subcutaneous injections of whole bee venom and its fractions on serum corticosterone concentrations are shown in Fig. 3. Serum corticosterone levels were determined in rats with adjuvant arthritis (Day 20) 1 and 4 hrs after the subcutaneous injection of the appropriate compounds. Whole bee venom produced sustained elevations of corticosterone, whereas melittin or phospholipase A alone each produced late and modest elevations. Apamin treatment resulted in a gradual progressive increase in cortico-

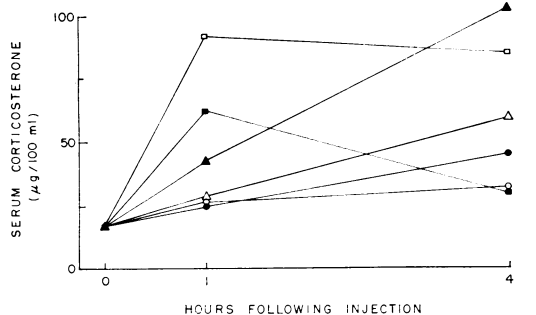


FIG. 3 Effect of whole bee venom and its fractions on serum corticosterone levels. Each point represents mean serum corticosterone concentration in five rats with adjuvant arthritis (Day 20 of disease). □: Whole bee venom subcutaneously 500 μ g.; ■: Whole bee venom intraperitoneally 375 μ g.; △: Melittin 250 μ g.; ▲: Apamin 50 μ g.; ●: Phospholipase A 80 μ g.; ○: Saline

Table I *Effect on blood leucocyte count, haematocrit, and weight of bee venom treatment in rats with adjuvant arthritis**

Time of measurement	Pre-treatment	Day 20	
		Control	Bee venom treatment
Blood leucocytes per mm ³ .	22,800	31,400	23,900
Small lymphocytes per mm ³ .	12,400	16,500	13,100
Haematocrit	44.7	39.4	44.0
Weight (g.)	254	245	275

* Values are means of six rats

Table II *Haemagglutinin titres in rats injected intraperitoneally with sheep red blood cells*

Treatment	No. of rats	No. of rats showing haemagglutinin titres of:			
		1/2,560*	1/5,120	1/10,240	1/20,480
Whole bee venom	5	1	2	2	0
Melittin	5	1	2	2	0
Apamin	5	0	3	2	0
Phospholipase A	5	0	2	3	0
Saline	5	0	0	0	5

* No agglutination at omitted lower titres

sterone concentrations, which by 4 hrs was comparable to the level produced by whole venom. Intraperitoneal injection of 375 µg. whole venom resulted in a modest transient elevation of corticosterone.

To study further the role of adrenal stimulation in the ameliorative action of bee venom, experiments were done with adrenalectomized rats. In contrast to our experience with prostaglandin E₁, injections of bee venom did not suppress adjuvant arthritis in adrenalectomized animals (Fig. 4). Adrenalectomized animals did not tolerate the optimal dose of bee venom and were treated with 300 µg. twice daily. Treatment was delayed until Day 3 in order to reduce stress to rats at time of adjuvant injection. Control animals died rapidly after the onset of polyarthritis because of their inability to maintain their intake of salt despite the fact that food, NaCl solution, and salt blocks were all placed in the cages. In contrast, rats treated with bee venom had joint scores comparable to the controls, but they were a little more active and able to continue their intake of salt. Arthritis was suppressed in PGE₁-treated rats, but mortality in this group was substantial even when rats were given 1.2 per cent. NaCl solution to drink.

Discussion

The polyarthritis of adjuvant disease in the rat has been regarded as a model for tissue injury mediated by delayed hypersensitivity (Waksman, Pearson, and Sharp, 1960; Pearson and Wood, 1964), and has been

used extensively for evaluating anti-inflammatory and immunosuppressive drugs (Newbould, 1963; Kalliomäki, Sääräma, and Toivanen, 1964; Winter and Nuss, 1966). Treatment of rats with whole bee venom (subcutaneously not intraperitoneally), but not with its major constituents alone, provided a high degree of protection against this disease, while producing no obvious side-effects. Bee venom, but not the individual fractions (nor bee venom given intraperitoneally), produced early and persistent rises in serum cortico-

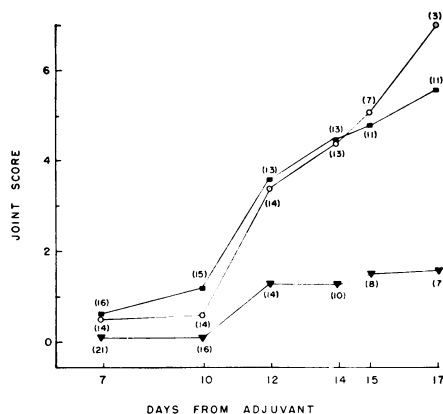


FIG. 4 *Effect on adjuvant arthritis in adrenalectomized rats of prostaglandin E₁ and whole bee venom. Numbers in parentheses indicate numbers of experimental animals remaining alive. ▼: PGE₁ 300 µg. subcutaneously twice daily (Days 3–16); ■: Whole bee venom 300 µg. subcutaneously twice daily (Days 3–16); ○: Controls*

sterone concentrations. Bee venom treatment does not prevent occurrence of disease in adrenalectomized rats, suggesting that the mechanism by which bee venom suppresses adjuvant disease is mediated through stimulation of the pituitary and/or adrenal glands. Whole bee venom also produces sustained (5 hrs) rises of plasma cortisol in dogs and monkeys, whereas hypophysectomy prevents bee venom-induced adrenal stimulation in these animals (Vick, Mehlman, and Shipman, 1971; Vick and Shipman, 1972). This ACTH-like effect of bee venom may account for the folk wisdom which maintains that bee sting and bee venom therapy afford protection against rheumatoid arthritis. Arguments against this interpretation can be raised, however, and other mechanisms which might account for bee venom action must be considered. For example, in rats treated with paramethasone, polyarthritis developed within 7 days after treatment was stopped (Newbould, 1963). Such an 'escape' phenomenon was not seen after treatment with whole bee venom. Adrenalectomized rats, not able to tolerate the high dose of bee venom given intact rats (500 μg . twice daily), were treated with 300 μg . twice daily. Although the lower dose did result in high levels of serum corticosterone in non-adrenalectomized rats, suppression of adjuvant disease was not as complete or consistent as that observed in rats treated with 500 μg . bee venom twice daily. It is possible that a portion of the bee venom effect is not related to adrenal gland stimulation and is achieved only at the high dose level. In addition, lymphocytopenia, a usual concomitant of adrenal stimulation in rats (Dougherty, Berliner and Berliner, 1960), was not observed after treatment with bee venom. Moreover, the antibody response to injected sheep red blood cells was modestly reduced by bee venom treatment. A more marked reduction in humoral antibody response (with preservation of

the cellular immune response to PPD) has been documented in adjuvant arthritic rats treated with prostaglandin E_1 (PGE_1) and it has been suggested that PGE_1 effects a restricted population of lymphocytes (Zurier and Quagliata, 1971). It is conceivable that a subtle interference with the immunological competence of lymphocytes (not related to adrenal stimulation) may be important to the mechanism(s) whereby bee venom suppresses adjuvant arthritis. Finally, bee venom therapy did not alter the course of established arthritis. Adrenocortical stimulation would be expected to modify the disease. Treatment of arthritic rats with dexamethasone (0.03 or 0.1 mg./kg./day p.o. for 5 days) resulted in 26 to 29 per cent. reduction in hind paw weight and a 32 to 43 per cent decrease in joint scores (Baruth, H. W., unpublished observations).

Summary

When rats are treated with whole bee venom, adjuvant arthritis is prevented. Similar treatment with the major known fractions of bee venom (apamin, melittin, phospholipase A) does not suppress disease. Although bee venom treatment has no effect on numbers of peripheral lymphocytes, it does cause modest reduction in the antibody response to injected sheep red blood cells. Whole bee venom, but not its constituents alone, produces sustained elevation of serum corticosterone concentrations. In addition, bee venom therapy does not prevent adjuvant arthritis in adrenalectomized rats, indicating that disease suppression may be mediated *via* the pituitary-adrenal axis. In contrast, treatment of adrenalectomized rats with prostaglandin E_1 (PGE_1) does protect against polyarthritis, suggesting that the effects of PGE_1 and bee venom on adjuvant disease are not necessarily related.

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