Biochemical Pharmacology, Vol. 31, No 6, pp. 1139–1146, 1982.
Printed in Great Britain.

0006-2952/82/061139-08 $03.00/0
Pergamon Press Ltd.

EFFECT OF HONEYBEE (*APIS MELLIFERA*) VENOM ON THE COURSE OF ADJUVANT-INDUCED ARTHRITIS AND DEPRESSION OF DRUG METABOLISM IN THE RAT

JULIE L. EISEMAN, JURGEN VON BREDOW and ALVITO P. ALVARES*
Department of Pharmacology, Uniformed Services University, Bethesda, MD 20814, U.S.A.

(*Received* 21 *April* 1981; *accepted* 4 *September* 1981)

Abstract—The ability of honeybee venom to suppress *Mycobacterium butyricum*-induced arthritis was studied in Lewis rats. Bee venom, 2 mg·kg^{-1}·day^{-1} for 24 days, suppressed but did not abolish the primary and secondary inflammatory responses to the adjuvant as monitored by decreases in the swelling of the left and right hind paws respectively. Bee venom itself caused no swelling of the hind paws. The effects of bee venom and adjuvant-induced arthritis on heme metabolism were also examined. Bee venom or adjuvant had no effect on hepatic δ-aminolevulinic acid synthase, porphyrin content, or ferrochelatase activity. However, with both treatments cytochrome P-450 and the associated enzymic activities of ethylmorphine *N*-demethylase and benzo[*a*]pyrene hydroxylase were depressed markedly. In contrast, both treatments caused several-fold enhancement of hepatic microsomal heme oxygenase activity. Adjuvant-treated rats receiving bee venom showed changes in heme metabolism which were of a magnitude similar to those observed when either agent was administered to the experimental animals. Although the bee venom appears to suppress adjuvant-induced arthritis to a greater extent in female than in male rats, the alterations in heme metabolism were similar in bee venom-treated male and female rats. The observed changes in heme metabolism elicited by the venom or by the adjuvant are strongly suggestive of perturbations of the immune system causing alterations in hepatic microsomal enzymes.

The venom of the honeybee (*Apis mellifera*) is comprised primarily of phospholipase A and melittin, a complex protein. These two components account for 85% of the total chemical constituents of the bee venom. Bee venom therapy has been reported to be effective in the treatment of rheumatoid arthritis in humans [1], as well as in experimental animals [2]. Bee venom, administered subcutaneously, suppressed carrageenan-induced paw edema and adjuvant-induced arthritis in the rat [2]. The venom, but not the individual components, when administered subcutaneously, produces early and persistent increases in corticosterone concentrations [3]. Whole bee venom has also been shown to produce a sustained increase in plasma cortisol levels in dogs and monkeys, whereas hypophysectomy prevents bee venom-induced adrenal stimulation in these animals [4, 5].

Adjuvant-induced disease in the rat includes a severe and persistent polyarthritis, which appears 10–14 days after a single intradermal injection of complete Freund's adjuvant and is widely accepted as a useful animal model of the human arthritic disease [6]. Induction of adjuvant arthritis in rats is accompanied by a marked decrease in cytochrome P-450-dependent monooxygenases. Previous studies have shown that the activity of these enzymes decreases markedly within the first 24 hr after the administration of Freund's adjuvant and stays depressed throughout the entire inflammatory response [7, 8]. This rat model responds to a wide variety of anti-rheumatic drugs. Aspirin and prednisolone produce complete abolition of the secondary inflammatory response, whereas phenylbutazone, gold, and other non-steroidal anti-inflammatory agents produce a marked suppression of this response [9–11]. In this study, we have re-evaluated the effect of bee venom therapy on adjuvant-induced arthritis. In addition, since a major fraction of the heme that is synthesized in the liver serves as a prosthetic group for cytochrome P-450, the terminal oxidase in the biotransformation of a variety of drugs, carcinogens and other foreign chemicals, we have examined the relationship between the effect of honeybee venom on adjuvant-induced arthritis and on heme and drug metabolism in the rat.

MATERIALS AND METHODS

Animals. Male and female Lewis rats weighing 120–150 g were used in these studies. Crystalline bee venom was a gift from Mr. Charles Mraz of the Warren Memorial Arthritis Foundation. Bee venom solutions were prepared fresh weekly and administered subcutaneously on the back of the rats. Rats were divided into four treatment groups: group 1, serving as controls, received daily subcutaneous injections of the vehicle beginning on day zero; group 2 received bee venom administered subcutaneously; group 3 received a single intradermal injection of 0.25 mg of *Mycobacterium butyricum* (Freund's adjuvant), suspended in mineral oil, into the left hind paw on day zero; and group 4 received the same intradermal dosage of Freund's adjuvant and, in

* To whom reprint requests should be addressed.

Table 1. Effect of chronic bee venom treatment on hepatic heme biosynthesis and drug metabolism in the Lewis rat*

Assay	Controls	Bee venom dosage ($mg \cdot kg^{-1} \cdot day^{-1}$)				
		0.010	0.033	0.100	0.330	1.000
ALA synthase (nmoles ALA formed $\cdot g^{-1} \cdot hr^{-1}$)	14.87	33.41	24.99	23.60	32.07	27.39
Porphyrin content (pmoles/mg protein)	9.73 ± 1.14	7.67 ± 0.59	6.27 ± 1.11	7.36 ± 0.95	7.69 ± 0.30	7.20 ± 0.30
Ferrochelatase [nmoles mesoheme formed $\cdot$ (mg protein)$^{-1} \cdot hr^{-1}$]	8.96 ± 0.91	6.64 ± 0.40	5.41 ± 0.97	7.93 ± 0.86	6.17 ± 0.74	6.72 ± 0.44
NADPH–cyt. *c.* reductase [nmoles cyt. *c.* reduced $\cdot$ (mg protein)$^{-1} \cdot min^{-1}$]	85.4 ± 4.7	66.1 ± 9.4	69.8 ± 12.4	88.3 ± 0.5	71.6 ± 8.0	57.4 ± 9.2†
Cytochrome P-450 (nmoles/mg protein)	1.07 ± 0.10	0.61 ± 0.12†	0.61 ± 0.09†	0.66 ± 0.06†	0.58 ± 0.07†	0.67 ± 0.09†
Benzo[*a*]pyrene hydroxylase [nmoles OHBP formed $\cdot$ (mg protein)$^{-1} \cdot hr^{-1}$]	7.96 ± 0.72	4.23 ± 1.38†	4.60 ± 0.77†	5.72 ± 0.71†	3.85 ± 1.20†	3.21 ± 0.60†
Ethylmorphine *N*-demethylase [nmoles HCHO formed $\cdot$ (mg protein)$^{-1} \cdot hr^{-1}$]	260.1 ± 8.7	165.4 ± 35.7†	178.4 ± 42.9†	177.8 ± 21.8†	201.4 ± 19.3†	206.0 ± 10.6†
Microsomal heme oxygenase [nmoles bilirubin formed $\cdot$ (mg protein)$^{-1} \cdot hr^{-1}$]	1.423 ± 0.118	4.143 ± 1.025†	3.236 ± 0.123†	3.609 ± 0.341†	3.296 ± 0.498†	3.391 ± 0.211†

* Male Lewis rats received bee venom subcutaneously at the dosages indicated, daily for 24 days, and were killed on day 25. Each value except the ALA synthase values is the mean ± S.E. of at least four animals; ALA synthase values are the means of two rats.

† Value is significantly different from the respective control value ($P < 0.05$).

addition, received daily subcutaneous injections of bee venom solutions. Dosages of bee venom are indicated in the text. Body weights were recorded, and hind paw diameters were determined using a micrometer. Animals were killed by cervical dislocation 24 hr after the last dose of bee venom or its vehicle.

Tissue preparation. Rat livers were perfused *in situ* with 50 ml of isotonic KCl, excised, blotted, and weighed. They were then homogenized in 1.15% KCl, such that each milliliter contained the equivalent of 250 mg of liver, wet weight. The homogenate was then centrifuged at 9000 *g* for 20 min. The supernatant fraction was then centrifuged at 105,000 *g* for 1 hr. The 105,000 *g* supernatant fraction was saved as a source of biliverdin reductase for heme oxygenase assays. The microsome pellet obtained was resuspended in 0.1 M potassium phosphate buffer, pH 7.4, such that each milliliter contained microsome equivalent to 100 mg of liver, wet weight. Protein content of the various cell fractions was determined by the method of Lowry *et al.* [12] using bovine serum albumin as a standard.

Enzyme assays. δ-Aminolevulinic acid (ALA) synthase was determined by the method of Sassa *et al.* [13], using liver homogenate equivalent to 37.5 mg of liver, wet weight. Porphyrin content of liver was determined using homogenate equivalent to 2 mg tissue by the fluorometric method of Granick *et al.* [14], using coproporphyrinogen as a standard. Ferrochelatase activity was assayed by a modification of the method of Porra and Jones [15], as described by Eiseman and Alvares [16]. Benzo[*a*]pyrene hydroxylase activity was determined using the 9000 *g* supernatant fraction, equivalent to 4 mg of liver, wet weight, as described previously [17]. The phenolic products formed were measured fluorometrically by the method of Nebert and Gelboin [18] using 3-hydroxybenzo[*a*]pyrene as a standard. Ethylmorphine *N*-demethylase activity was determined as described earlier by Alvares and Mannering [19], except that nicotinamide was deleted from the incubation mixture. NADPH–cytochrome *c* reductase activity was determined by the method of Phillips and Langdon [20]. Cytochrome P-450 content was determined using the CO-difference spectrum of liver microsomes by the method of Omura and Sato [21]. Microsomal heme oxygenase activity was determined as described previously [16].

RESULTS

Effect of chronic bee venom administration to Lewis rats on heme metabolism. To determine the effect of bee venom on heme biosynthesis and catabolism, and on the monooxygenase system in the liver, male Lewis rats were treated with the venom at various dosages, daily for 24 days. The dosages are indicated in Table 1. The experimental animals were killed on day 25, and livers were removed and assayed for certain key enzymic activities. Following chronic, subcutaneous, administration of the bee venom, there were no statistically significant changes in any of the measured variables of hepatic heme biosynthesis. ALA synthase, the first and rate-limiting enzyme in the heme biosynthetic pathway, was slightly higher in all of the groups receiving bee venom. Total hepatic porphyrin content, as well as ferrochelatase, the final enzyme in the heme biosynthetic pathway, were not altered. A component of the monooxygenase system, NADPH–cytochrome *c* reductase activity, was decreased significantly only at the highest dose administered. Cytochrome P-450 contents were decreased significantly by 30–50% in all groups receiving bee venom. Concomitant with the decrease in the hemeprotein levels, there were significant decreases in the cytochrome P-450-dependent enzymic activities of

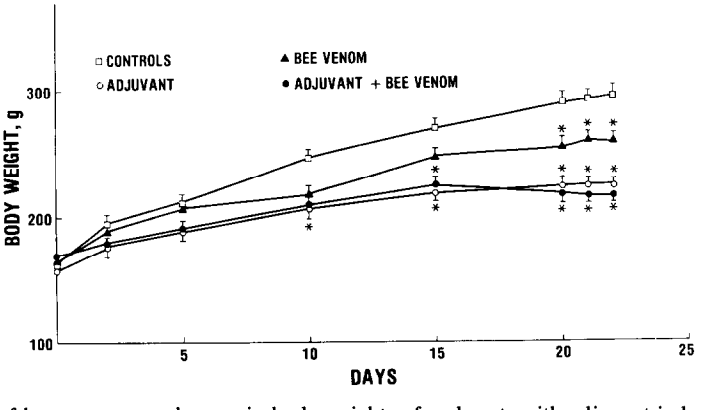

Fig. 1. Effect of bee venom on changes in body weights of male rats with adjuvant-induced arthritis. Key: (□) control rats received daily subcutaneous injections of saline, 0.5 ml/100 g body wt, and a single intradermal injection of 0.05 ml mineral oil into the left hind paw on day zero; (○) adjuvant-treated rats received a single intradermal injection of 0.25 mg adjuvant in 0.05 ml mineral oil into the left hind paw on day zero, and daily subcutaneous injections of saline, 0.5 ml/100 g body wt; (▲) bee venom-treated rats received subcutaneously the venom at a dose of 2.0 mg · kg^{-1} · day^{-1} for 24 days, and a single intradermal injection of 0.05 ml mineral oil into the left hind paw on day zero; and (●) adjuvant, 0.25 mg in 0.05 ml mineral oil, was injected intradermally into the left hind paw on day zero, and bee venom subcutaneously, 2.0 mg · kg^{-1} · day^{-1} for 24 days. Each value is the mean ± S.E. of at least four rats per group. An asterisk (*) represents a value significantly different from the respective control value ($P < 0.05$).

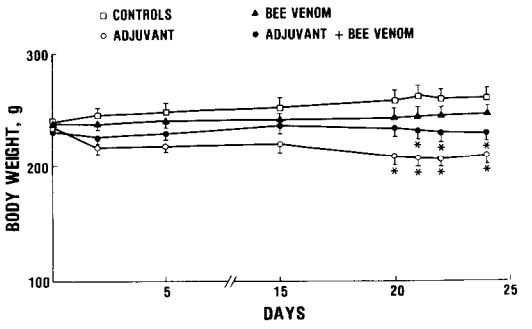

Fig. 2. Effect of bee venom on changes in body weights of female rats with adjuvant-induced arthritis. See legend to Fig. 1 for description of experimental protocol and symbols. Each value is the mean ± S.E. of at least four rats per group. An asterisk (*) represents a value significantly different from the respective control value ($P < 0.05$).

benzo[*a*]pyrene hydroxylase and ethylmorphine *N*-demethylase. Benzo[*a*]pyrene hydroxylase activity was decreased to 40% of control activity, whereas *N*-demethylase activity was decreased by only 20% of control activity, in rats receiving 1.0 mg/kg bee venom. As shown in Table 1, the activity of microsomal heme oxygenase, the rate-limiting enzyme in heme catabolism, was increased 2- to 3-fold in the livers of all the bee venom-treated animals. The observed alterations in enzymic activities did not appear to be dependent on the dose of the venom administered to the rats. Thus, chronic exposure of experimental animals to honeybee venom caused no significant effect on heme biosynthesis, but it did cause a significant decrease in drug-metabolizing enzymic activities and a concomitant increase in hepatic heme oxygenase activity.

Effect of bee venom therapy on the course of adjuvant-induced arthritis in male and female rats. Since the dose–response studies showed that bee venom had a significant effect on hepatic monooxygenases, and since bee venom has been found [1, 2] to be efficacious in the treatment of arthritis, it was important to study the interaction between bee venom treatment and adjuvant-induced arthritis. Morton and Chatfield [22] had previously shown a depression in *N*-demethylase activity and a decrease in the ability of the liver to form β-glucuronide conjugates in adjuvant-induced arthritic rats. In the present studies, Lewis rats were administered Freund's adjuvant into the left hind paw. Rats receiving the adjuvant were then divided into two groups, one receiving bee venom subcutaneously, $2.0\,mg \cdot kg^{-1} \cdot day^{-1}$, for 24 days and killed on day 25, the other receiving the vehicle, isotonic NaCl. A fourth group of rats received bee venom only. At dosages lower than $2\,mg \cdot kg^{-1} \cdot day^{-1}$, in data not shown, no significant effect on adjuvant-induced arthritis was observed. Body weights and hind paw diameters were monitored throughout the course of the experiment.

The changes in body weights in male and female rats are shown in Figs. 1 and 2 respectively. In confirmation of previous studies [23], and from data obtained from growth charts provided by Charles River Breeding Laboratories, the untreated female rats gained less body weight than the males during the 25-day experimental period. As expected, adjuvant-treated arthritic rats gained significantly less weight than did the controls. Arthritic rats receiving bee venom also demonstrated lowered body weights, particularly after initiation of the secondary response, which normally occurs 10–14 days after inoculation of Freund's adjuvant [24]. Body weights of adjuvant-treated rats did not differ sig-

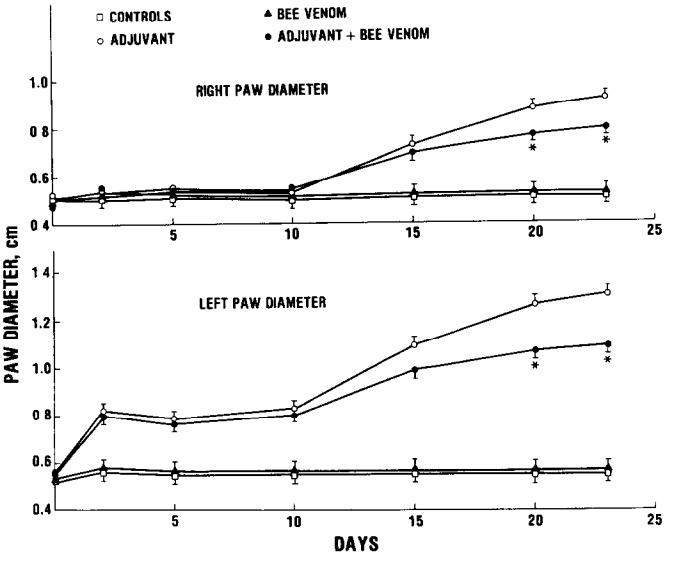

Fig. 3. Effect of bee venom on changes in hind paw diameters of male rats with adjuvant-induced arthritis. See legend to Fig. 1 for description of experimental protocol and symbols. An asterisk (*) represents a value significantly different from the respective value obtained with rats receiving adjuvant only ($P < 0.05$).

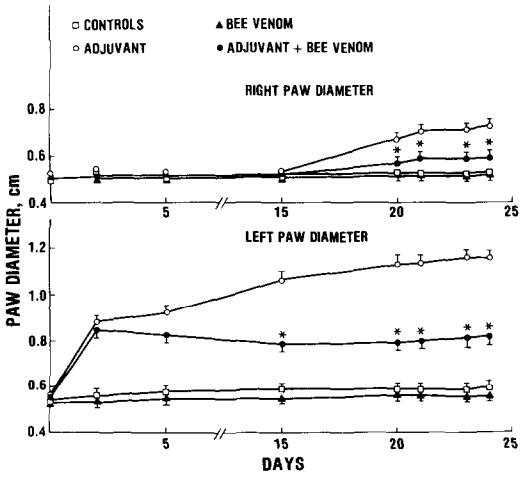

Fig. 4. Effect of bee venom on changes in hind paw diameters of female rats with adjuvant-induced arthritis. See legend to Fig. 1 for description of experimental protocol and symbols. An asterisk (*) represents a value significantly different from the respective value obtained with rats receiving adjuvant only ($P < 0.05$).

nificantly from body weights of adjuvant rats also receiving bee venom (Figs. 1 and 2).

Injection of the adjuvant in the left hind paw of the rat caused a "primary" swelling of the injected foot with a maximum response occurring 2–3 days after injection. The "secondary" response, which occurred 10–14 days after injection, was manifested by swelling of the non-injected right hind paw and tail. The forepaws are usually less affected than the hind paws and the tail [25]. Hind paw diameters of male and female rats were recorded in untreated, bee venom-treated, and arthritic rats that received or did not receive bee venom, 2 mg · kg^{-1} · day^{-1} for 24 days. The data for the male and female rats are shown in Figs. 3 and 4 respectively. The primary response to the adjuvant, as determined by an increase in the left hind paw diameter, was evident in the adjuvant rats as well as the adjuvant rats receiving bee venom. Bee venom caused a significant decrease in both the mean right and left hind paw diameters in the arthritic rats during the secondary response. The beneficial effect of bee venom appeared to be more pronounced in the female rat (Fig. 4) than in the male rat (Fig. 3). The paw

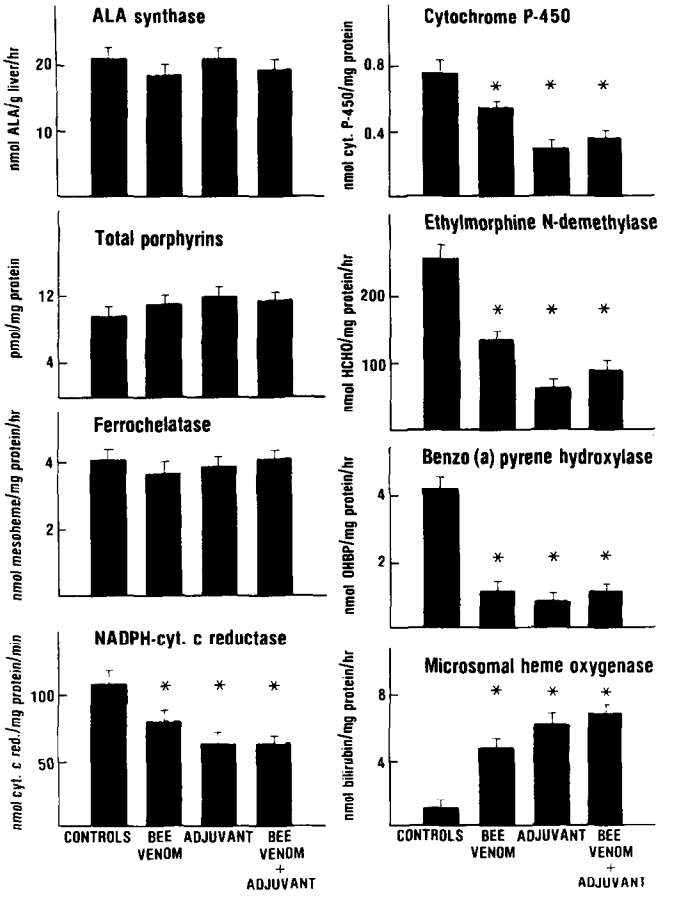

Fig. 5. Effect of bee venom on hepatic heme metabolism in control and adjuvant-induced arthritic male rats. Animals were treated as described in the legend to Fig. 1 for 24 days. Rats were killed on day 25 and livers were assayed. Each bar is the mean ± S.E. of at least four rats per group. An asterisk (*) represents a value significantly different from the control value ($P < 0.05$).

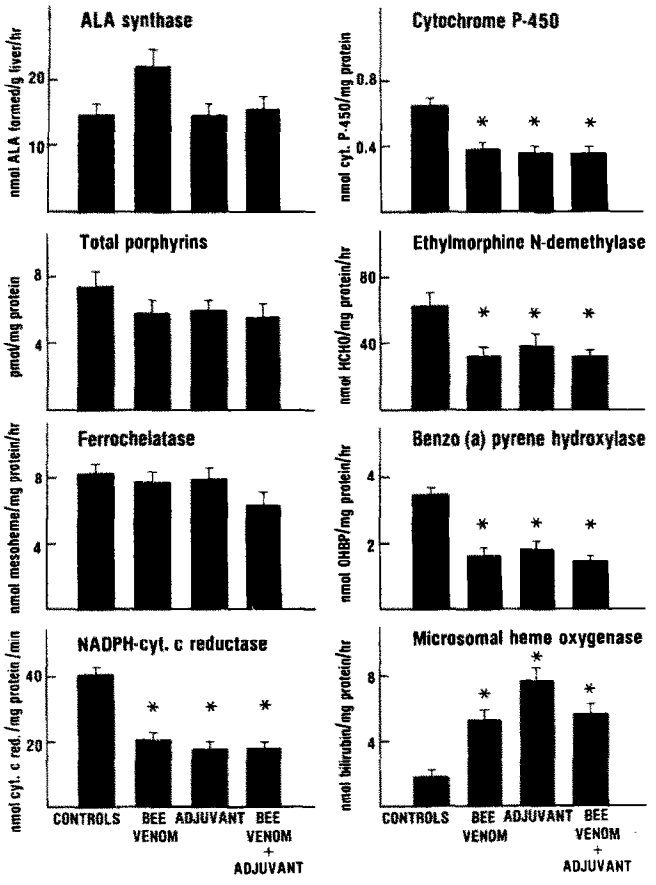

Fig. 6. Effect of bee venom on hepatic heme metabolism in control and adjuvant-induced arthritic female rats. Rats were treated as described in the legend to Fig. 1 for 24 days. Rats were killed on day 25 and livers were assayed. Each bar is the mean ± S.E. of at least four rats per group. An asterisk (*) represents a value significantly different from the control value ($P < 0.05$).

diameters of rats receiving bee venom only were similar to the paw diameters of the control rats receiving the vehicle. Thus, these data demonstrate that the bee venom possesses the ability to suppress, but not to abolish, the inflammatory response elicited by adjuvant administration.

Effect of bee venom administration on heme metabolism in the livers of adjuvant-pretreated rats. Bee venom was administered to control rats and to rats that received Freund's adjuvant on day zero. The venom was administered subcutaneously daily for 24 days, and rats were killed 24 hr after the last injection. Heme biosynthesis and catabolism, and the functional capacity of the cytochrome P-450 system, were determined in the livers of the male (Fig. 5) and female (Fig. 6) rats. Bee venom administered to control rats or arthritic rats had no effect on ALA synthase and ferrochelatase activities or porphyrin concentrations in the heme biosynthetic pathway in the liver. However, both the adjuvant and bee venom caused significant decreases in NADPH–cytochrome *c* reductase activities and cytochrome P-450 content of liver microsomes. As a consequence, ethylmorphine *N*-demethylase and benzo[*a*]pyrene hydroxylase activities were decreased markedly. Microsomal heme oxygenase activity was enhanced 4- to 6-fold in the male rat (Fig. 5) and 2- to 3-fold in the female rat (Fig. 6). In all variables measured, adjuvant-treated rats receiving bee venom showed changes which were of a magnitude similar to those observed when either agent was administered to the male or female rat.

DISCUSSION

Adjuvant arthritis is inducible following the injection of a fine dispersion of a heat-killed *M. butyricum* in mineral oil into the footpad of a rat. Not all strains of rats are susceptible to the adjuvant. The Lewis strain of rats has been reported to be a particularly good responder [7], and studies from several laboratories have shown impaired drug metabolism in adjuvant-induced arthritic rats [7, 8, 22]. Other evidence implicating hepatic involvement includes a reduction in serum albumin [26] and a rise in circulating fibrinogen [27]. Studies by Cawthorne *et al.* [8], and Whitehouse and Beck [7], have shown that, in these rats, the impairment of the drug-metabolizing enzymes is not necessarily dependent on the

development of arthritic lesions, indicating that the two phenomena may not necessarily be related.

Recent studies [3] have indicated that bee venom suppresses *Mycobacterium tuberculosis*-induced arthritis in rats. Our studies demonstrate that bee venom did have a suppressive effect on adjuvant-induced arthritis, but the time–course studies showed that this was a delayed response. The suppressive effects were statistically significant only at 2–3 weeks following the administration of the adjuvant to the rats. Further, there appeared to be a sex difference in the suppressive effect of bee venom on the adjuvant arthritis, as determined by monitoring of the hind paw diameters.

The clinical relevance of bee venom therapy in the treatment of human rheumatoid arthritis is questionable. The dosage of the venom employed in our studies, 2 mg/kg, was rather high. At lower dosages, in data not shown, no significant suppressive effect on adjuvant arthritis was noted. The average honeybee sting contains about 50 μg solids [28]. A recent study of active beekeepers who received a mean of 10 stings per day showed no gross changes in blood chemistry or incidence of urine abnormalities [29]. In a recent study, Billingham *et al.* [30] reported the isolation of a polypeptide from honeybee venom which was found to be 100 times more active than hydrocortisone in inhibiting carrageenin-induced edema in paws of Wistar rats. This fraction of bee venom may be an effective anti-arthritic agent. Two major components of bee venom, melittin and phospholipase A, were shown previously to have no significant effect on adjuvant-induced polyarthritis [3].

It was of interest to note from data obtained in our studies that injection of bee venom or treatment of rats with adjuvant resulted in very similar effects on heme metabolism. Both agents had no significant effect on heme biosynthesis, even though a marked decrease in the hemeprotein cytochrome P-450 content was observed in the liver microsomal preparations of the treated rats. The decrease in hemeprotein content was accompanied by decreases in the cytochrome(s) P-450-associated enzymic activities of ethylmorphine *N*-demethylase and benzo[*a*]pyrene hydroxylase. Another major component of the hepatic monooxygenase system is NADPH–cytochrome *c* reductase, also known as cytochrome P-450 reductase. In both male and female rats, bee venom treatment or induction of adjuvant arthritis caused a 40–50% decrease in the activity of this flavoprotein. It would be of interest to determine if beekeepers, who are chronically stung by honeybees, or patients with rheumatoid arthritis, have impaired drug-metabolizing capacities, particularly those reactions which are dependent on cytochrome P-450. Our present data also indicate that administration of bee venom to adjuvant-treated rats did not enhance the effects observed when either agent was administered alone. Further, the decrease in heme content as reflected by a decrease in cytochrome P-450 content did not, under our experimental conditions, result in an induction of ALA synthase, the rate-limiting enzyme in the heme biosynthetic pathway. It is possible that a transient elevation of ALA synthase may have occurred initially and the activity of this enzyme may have returned to control levels by the time the animals were killed.

The decrease in the hemeprotein content may have been related to a marked increase in microsomal heme oxygenase observed with liver obtained from rats treated with bee venom, adjuvant, or both agents. The enzyme, which catalyzes the metabolism of heme to biliverdin, was enhanced 4- to 6-fold in the male rat and 2- to 3-fold in the female rat. Previous studies have shown that endotoxins stimulate heme oxygenase in the liver [31, 32], block stimulation of ALA synthase by porphyrinogenic agents [32, 33], and exert an adjuvant-like effect on antibody response [34]. Since phospholipases *in vitro* inhibit monooxygenases and inactivate cytochrome P-450 to P-420 [35], the possibility exists that the decreases in cytochrome P-450 and associated enzymic activities observed with bee venom-treated rats may have been due to the administration of phospholipase A, a major component of bee venom [3]. Endotoxin has also been shown to be a potent depressor of cytochrome(s) P-450 [36]. Endotoxins also induce interferon [37]. Antigens, mitogens, or antigen–antibody complexes may stimulate immune (type II) interferons, and interferons have also been found in the sera of patients with rheumatoid arthritis [38]. In addition to causing decreases in cytochrome P-450 and associated enzymic activities, interferon-inducing agents have been shown to cause a marked increase in microsomal heme oxygenase within the 24 hr following the administration of an interferon-inducing agent [39]. The depression of cytochrome P-450-dependent enzymic activities by venom antigens or by *M. butyricum* may have been due to a perturbation of one or more components of the immune system. Barnes *et al.* [40] have suggested previously that decreased hepatic monooxygenase activity may be a common property of all immunomodulators.

Acknowledgements—The authors thank Miss Jane F. Neubauer for her technical assistance and Mr. Mark O. Chapman for his secretarial help in the preparation of this manuscript.

REFERENCES

1. B. F. Beck, *Bee Venom Therapy: Bee Venom, Its Nature and Its Effect on Arthritic and Rheumatoid Conditions*, pp. 171–197. Appleton-Century Croft, New York (1935).
2. Y-H. Chang and M. L. Bliven, *Agents Actions* **9**, 205 (1979).
3. R. B. Zurier, H. Mitnick, D. Bloomgarden and G. Weissmann, *Ann. rheum. Dis.* **32**, 466 (1973).
4. J. A. Vick, B. Mehlman and W. H. Shipman, *Pharmacologist* **13**, 240 (1971).
5. J. A. Vick and W. H. Shipman, *Toxicon* **10**, 377 (1972).
6. C. M. Pearson, *Proc. Soc. exp. Biol. Med.* **91**, 95 (1956).
7. M. W. Whitehouse and F. J. Beck, *Drug Metab. Dispos.* **1**, 251 (1973).
8. M. A. Cawthorne, E. D. Palmer and J. Green, *Biochem. Pharmac.* **25**, 2683 (1976).
9. J. R. Ward and R. S. Clud, *J. Pharmac. exp. Ther.* **152**, 116 (1966).
10. B. B. Newbould, *Br. J. Pharmac.* **21**, 127 (1963).

11. D. T. Walz, M. J. DiMartino and A. Misher, *Ann. rheum. Dis.* **30**, 303 (1971).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
13. S. Sassa, A. Kappas, S. E. Bernstein and A. P. Alvares, *J. biol. Chem.* **254**, 729 (1979).
14. S. Granick, P. Sinclair, S. Sassa and P. Grieninger, *J. biol. Chem.* **250**, 9215 (1975).
15. R. J. Porra and O. T. G. Jones, *Biochem. J.* **87**, 181 (1963).
16. J. L. Eiseman and A. P. Alvares, *Molec. Pharmac.* **14**, 1176 (1978).
17. A. P. Alvares, D. R. Bickers and A. Kappas, *Proc. natn. Acad. Sci. U.S.A.* **70**, 1321 (1973).
18. D. W. Nebert and H. V. Gelboin, *J. biol. Chem.* **239**, 2370 (1968).
19. A. P. Alvares and G. J. Mannering, *Molec. Pharmac.* **6**, 206 (1970).
20. A. H. Phillips and R. G. Langdon, *J. biol. Chem.* **237**, 2652 (1962).
21. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
22. D. M. Morton and D. H. Chatfield, *Biochem. Pharmac.* **19**, 473 (1970).
23. P. P. Mathur, G. S. Carr and R. D. Smyth, *Biochem. Pharmac.* **27**, 1379 (1978).
24. C. M. Pearson, *Arthritis Rheum.* **7**, 80 (1964).
25. C. M. Pearson and F. D. Wood, *Arthritis Rheum.* **2**, 440 (1959).
26. E. M. Glenn, B. J. Bowman and T. C. Koslowske, *Biochem. Pharmac.* (Suppl.) 27 (1968).
27. E. M. Glenn, *Biochem. Pharmac.* **18**, 317 (1969).
28. L. M. Lichtenstein, M. D. Valentine and A. K. Sobotka, *New Engl. J. Med.* **290**, 1123 (1974).
29. J. W. Yungiger, R. T. Jones, K. M. Leiferman, B. R. Paull, P. W. Welsh and G. J. Gleich, *J. Allergy clin. Immun.* **61**, 93 (1978).
30. M. E. J. Billingham, J. Morley, J. M. Hanson, R. A. Shipolini and C. A. Vernon, *Nature, Lond.* **245**, 163 (1973).
31. D. Gemsa, C. H. Woo, H. H. Fudenberg and R. Schmid, *J. clin. Invest.* **53**, 647 (1974).
32. D. M. Bissell and L. E. Hammaker, *Archs Biochem. Biophys.* **176**, 91 (1976).
33. D. M. Bissell and L. E. Hammaker, *Archs Biochem. Biophys.* **176**, 103 (1976).
34. J. K. Spitznagel and A. C. Allison, *J. Immun.* **104**, 128 (1970).
35. M. D. Chaplin and G. J. Mannering, *Molec. Pharmac.* **6**, 631 (1970).
36. G. J. Mannering, K. W. Renton, R. el Azhary and L. B. Deloria, *Ann. N.Y. Acad. Sci.* **350**, 314 (1980).
37. J. S. Youngner and W. R. Stinebring, *Virology* **1**, 310 (1966).
38. J. J. Hooks, H. M. Moutsopoulos and A. L. Notkins, *Ann. N.Y. Acad. Sci.* **350**, 21 (1980).
39. R. el Azhary and G. J. Mannering, *Molec. Pharmac.* **15**, 698 (1979).
40. D. W. Barnes, P. S. Morahan, S. Loveless and A. E. Munson, *J. Pharmac. exp. Ther.* **208**, 392 (1979).