Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

Contribution of Lymphocytes in Edema Induced by Venom from the Wasp (Belonogaster fuscipennis)

A.E. Eno, O.I. Owo, E.H. Itam & R.S. Konya

To cite this article: A.E. Eno, O.I. Owo, E.H. Itam & R.S. Konya (2001) Contribution of Lymphocytes in Edema Induced by Venom from the Wasp (Belonogaster fuscipennis), Pharmaceutical Biology, 39:4, 247-252, DOI: 10.1076/phbi.39.4.247.5912

To link to this article: https://doi.org/10.1076/phbi.39.4.247.5912

Pharmaceutical Biology
2001, Vol. 39, No. 4, pp. 247–252

1388-0209/01/3904-247$16.00

Contribution of Lymphocytes in Edema Induced by Venom from the Wasp (*Belonogaster fuscipennis*)

A.E. Eno[1], O.I. Owo[1], E.H. Itam[2] and R.S. Konya[3]

[1]Department of Physiology and [2]Biochemistry, College of Medical Sciences, University of Calabar, Nigeria, and [3]Department of Zoology, University of Portharcourt, Portharcourt

Abstract

Subplantar injection of *Belonogaster fuscipennis* crude venom (40 µg/paw) induced rat hind-paw edema. The edema was slow in onset and reached maximum size in the fifth hr after injection of the venom. In amethopterin-induced leucopenic rats, the edematogenic response to the venom (40 µg/paw) was significantly reduced. Lymphocytes suspension given i.v. to another group of leucopenic rats caused a reversal of this response, but neutrophils suspension failed to reduce the response. In animals rendered lymphocytopenic by experimental obstruction of the thoracic duct, the oedema-forming activity of the venom was also markedly reduced. From these results, we suggest that the circulating lymphocytes participate in *B. fuscipennis* venom-induced edema.

Keywords: Venom, *Belonogaster fuscipennis*, edema, lymphocytes.

Introduction

Belonogaster fuscipennis, (Schmidt et al., 2000) commonly known as "atat" in the Eastern part of Nigeria, is a social wasp found in most parts of West Africa. Among the many species of vespine wasps found in Nigeria, *B. fuscipennis* is probably the most dangerous. The high incidence of sting by this insect is due to its aggressive behaviour, especially when disturbed, since it builds its comb near houses in urban and rural areas. The *B. fuscipennis* sting is more prevalent than bee stings and snake bites in the rural areas or villages in some Eastern parts of Nigeria.

The major early feature of poisoning by animal venoms at the affected site is often local edema (Ho et al., 1993). Stings by hymenoptera such as hornets, yellow-jackets and honey bees are manifested by symptoms of pain, local edema and cardiovascular disturbances (Habermann, 1972; Pick, 1984).

The *B. fuscipennis* venom-induced edematogenic effect is time-and dose-dependent, and involves the participation of inflammatory mediators such as the kinins, autacoids and lipoxygenase derivatives (Eno, 1997). Little is known, however, about the contribution of haematogenous cells in the phenomenon, despite the fact that it has long been appreciated that leucocytes are relevant factors in inflamation. Inflammatory exudates include most, if not all, of the cells of the leucocytic series.

In addition, lymphocytes are thought to play a role in the development of acute inflammatory reactions. They are capable of releasing pro-inflammatory substances that are of importance for adequate response against injury (Garcia Leme, 1981; Garcia Leme et al., 1976; Bechara et al., 1976). Furthermore, surgical obtruction of the thoracic duct, with interruption of lymphocyte recirculation, is accompained by decreased lymphocyte counts in blood and reduced inflammatory reactions (Sudo & Garcia Leme, 1980).

In the present studies, we have attempted to investigate the role of leucocyte cells in the rat paw swellings induced by *B. fuscipennis* venom. This entailed the induction of leucopenia or lymphocytopenia in rats.

Materials and Methods

Evaluation of edema formation

Crude venom was collected by pressing the *B. fuscipennis* venom sacs dissected from the anaesthetized worker insect (Ho et al., 1993; Ho & Hwang, 1991). The venom was

Accepted: July 24, 2000

Address correspondence to: Dr A. E. Eno, Department of Physiology, College of Medical Sciences, University of Calabar, P. M. B. 1115, Calabar, Cross River State, Nigeria. E-mail: nkin@unical.anpa.net.ng

lyophilized and maintained at –20°C (Ho et al., 1993; Chaves et al., 1995).

Hind paw edema was induced by a single sulplantar injection of the venom (40 µg in 0.1 ml saline per paw) into the right hind paws of white Wistar rats (200–280 g body wt.) An equal volume of saline was injected into the left hind paw as control. The volume of each paw was measured with a plethysmometer (Ugo basile, Model 7150). The degree of paw-swelling was expressed as % increase of the initial volume. Paw swelling caused by the injection of saline (in the left paw) was substracted from the data in each experiment. Significance of the data was determined by Student's *t*-test.

Depletion of lymphocytes by the injection of amethopterin

The procedure employed was as described by Cury et al. (1994). Amethopterin, dissolved in saline (0.9% NaCl) was given i.p. to rats in doses of 2.5, 1 and 1 mg/kg at 24 hr intervals. The total as well as differential leucocyte counts were performed on the day on which drug administration started and 3 days after the last dose. For the study of the edematogenic effect of the venom, only animals with a reduction of about 50–75% in total leucocyte counts were used. In some experiments, leucopenic animals were injected i.v. before edema production, with lymphocyte or neutrophil suspension, in volumes not exceeding 0.8 mL. The difference between the initial count in the peripheral blood and the number determined after induction of leucopenia was used to estimate the number of cells injected, assuming total blood volume to be 10 mL.

Leucocyte counts

The blood samples were collected from the tail veins. The tails were massaged repeatedly and kept warm to increase blood flow to this area. The tips were cleaned with ethyl alcohol and then snipped with a clean scalpel. Blood samples were collected drop by drop into heparinized tubes, and then diluted (1 : 20) with Turk solution. Counts of the white cells were done in Neubauer chambers. In the differential counts studies, blood smears, thin enough to separate the cells were prepared on the microscope slides. The blood films were dried in air and then stained with Leishman's stain before counting under a light microscope.

Separation of lymphocytes

The method described by Garcia Leme et al. (1976) to obtain lymphocytes from normal (untreated) rat spleen was employed. Briefly, using forceps, the organ was reduced to a pulp and passed through a sieve with 0.2 mm diameter meshes. Phosphate-buffered saline (PBS) (equal volume pH 7.4) was added, and centrifuged for 5 min at 4°C. The supernatant was discarded and the sediment resuspended in 20 mL PBS. This washing procedure was repeated three times. The cells were then counted in Neubauer chambers and their viability estimated by the eosin-Y exclusion test. Lymphocyte suspensions containing 95% or more viable cells were acceptable for use.

Isolation of neutrophils

The neutrophils were isolated by the method of Cury et al. (1994). Normal albino white Wistar rats were injected i.p. with 1 mL of 2% glycogen solution in PBS. Four hours later, the animals were anaesthetized with ether. A midline incision was made at the abdomen, and the abdominal cavity rinsed with 20 mL PBS. The exudates were centrifuged for 5 min, the cells separated and their viability tested. About 92% of the cells present in the exudates were found to be neutrophils. Their number was estimated as described for lymphocytes isolation above.

Depletion of lymphocytes by thoracic duct obstruction

The animal was anaesthetized with ether. The thoracic duct was exposed and then ligated in its intra-abdominal course using silk thread. The ligatures were at two points close to each other above the cisterna chyli. The duct was then divided between the ligatures. Another group of animals (sham-operated) underwent the same surgical procedures except that the ducts were left untouched. Both groups of animals were then kept in cages for three days, with free access to food and water. After the three days duration of the experiment, they were given a single subplanter injection of 2.5% solution of Evans blue in the right hind paws. They were killed 20 min thereafter. The presence of the dye was not detected above the severed endings, in animals subjected to thoracic ducts ligation.

Statistical analysis

The results were statistically analysed by means of Student's *t*-test or two-way ANOVA, followed by the Bonderroni method (Wallenstein et al., 1980). P-values of 0.05 or less were considered significant.

Results

Influence of amethopterin treatment on leucocyte counts

The rats were given three separate doses of 2.5, 1.0 and 1.0 mg/kg amethopterin at 24 hr intervals. Leucocyte counts were performed before (initial) and three days after (final) the last dose. In untreated animals, an equivalent time interval between counts was observed. Results (Table 1) show that there was a significant decrease in the total number of circulating leucocytes in the blood following the administration of amethopterin. This decrease was up to 58.5 ± 8.4% (SEM,

Table 1. Effect of amethopterin administration on leucocyte counts in the rat.

Animal Groups	Total Leucocytes (per ml)	Differential Counts (Cells/ml)				
		Lymphocytes	Neutrophils	Monocytes	Eosinophils	Basophils
Unitreated (n = 10)						
Initial	7989 ± 548	5323 ± 515	1510 ± 217	1296 ± 119	148 ± 43	8 ± 6
Final	9255 ± 602	6015 ± 473	1822 ± 149	1711 ± 182	126 ± 36	14 ± 9
Treated (n = 20)						
Initial	10186 ± 320	7356 ± 298	1488 ± 125	1328 ± 130	87 ± 35	5 ± 3
Final	4231 ± 285*	3298 ± 246*	1233 ± 38*	551 ± 78*	121 ± 42*	3 ± 1

Rats were injected with three separate doses of 2.5, 1.0 and 1.0 mg/kg amethopterin at 24 hr intervals. Leucocyte counts were performed before (initial) and 3 days after (final) the last does. The same time interval between counts was observed in the untreated animals. Results are the mean values ± S.E.M. *$p < 0.05$ vs. initial counts.

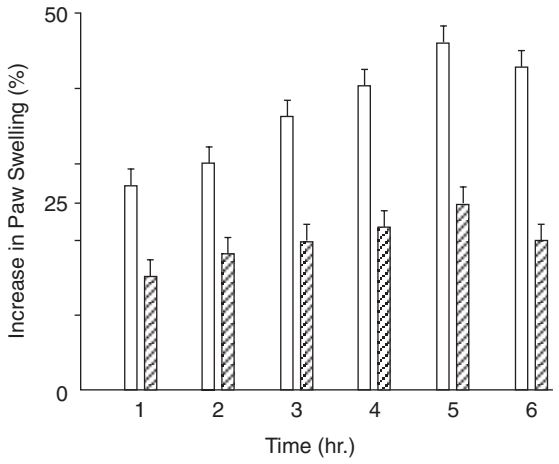

Figure 1. Time course of the edematogenic response of *B. fuscipennis* venom (40 µg/pw). Data are expressed as % increase in paw swelling as compared to control paws. Normal rats (open column); leucopenic rats (hatched column). Each point represents mean value ± S.E.M. n = 8.

n = 20, $p < 0.05$). Differential count studies revealed that all the white blood cells (polymorphonuclear granulocytes and monocytes) except the eosinophils were reduced by the drug treatment. The drug (amethopterin) produced about 55.2, 91.4, and 58.6% reduction in circulating lymphocytes, neutrophils and monocytes, respectively, when compared to their matching controls. Since lymphocytes are the prevailing white cells in the rat (Cury et al., 1994), a greater percentage of the reduction by amethopterin was due to a decrease in the number of circulating lymphocytes. The results (Table 1) therefore suggest that the animals were strictly leucopenic.

Venom-induced edema in leucopenic rats

In the normal (control) rats, intraplantar injection of *B. fuscipennis* venom (40 µg/paw) into the right hind paw resulted in edema which was very slow in onset and reaching a peak in about 5 hr, before fading slowly thereafter. At peak level of response, there was about 48% (% control) increase in paw swellings produced by the venom (40 µg/paw). In amethopterin-induced leucopenic rats, significant reductions in paw swellings were observed as compared with the normal rats (controls) (Fig. 1). The edematogenic response was reduced by about 46.8% (at peak level) in leucopenic rats and even greater reduction (54.6%) an hour later.

Effect of administering lymphocyte and neutrophil suspensions on the venom-induced edema in leucopenic rats

The inhibition of the venom-induced paw swellings in leucopenic rats, as compared with normal rats (Fig. 1), was reversed when the suspension of lymphocytes collected from the spleen were injected i.v. 20 min earlier into another group of leucopenic rats (Fig. 2). However, the injection of neutrophil suspension failed to reverse the edematogenic response in leucopenic rats. The volumes of both suspensions injected contained sufficient quantities of the particular cells (lymphocytes or neutrophils) to apparently counteract the deficiency produced by amethopterin treatment. Also, the i.v. injection of PMN granulocyte suspension collected from the peritoneal cavity 4 hr after i.p. injection of a 2% glycogen solution, attenuated the edematogenic response to the venom in leucopenic rats (Fig. 2).

Obstruction of the thoracic duct and leucocyte counts

Table 2 summarizes the effect of thoracic duct ligation, with the interruption of lymph circulation, on leucocyte counts in the rat. Leucocyte counts were performed before (initial) and three days after (final) the experimental obstruction of the thoracic duct. Equivalent time intervals were observed between initial and final counts for matching groups of sham-operated and normal animals. There was marked lymphopenia of about 52% (% control), but no effect on other cell types. In animals that received *B. fuscipennis* venom

(40 µg/paw) 3 days after thoracic duct ligation, the edema was markedly reduced (by about 23–72% between 1–6 hr) (Fig. 3).

The operated animals were tested for their capacity to respond to the venom on the third day after ligation of the thoracic duct. Lymphoedema did not develop in the paws following thoracic duct ligation.

Discussion

Inflammation is a protective response which serves to dilute, remove or destroy the inciting (toxic) agent in animals (Gallin et al., 1988). Its acute form is characterized by signs of erythema, fever, pain and edema, which are valuable in monitoring the progress of inflammation (Ho et al., 1993). Studies on the hornet wasp (*Vespa basalis*) venom have revealed that the enzyme protease (kininogenase) did not affect the edematous effect. Rather, serotonin components are responsible for the edema-inducing activity of the venom (Ho & Hwang, 1991). In previous studies, a conspicous dose-dependent edema was observed after the injection of *B. fuscipennis* venom in rat paws (Eno, 1997). The kinins, autacoids and lipoxygenase derivatives were some of the venom components involved in this reaction (Eno, 1997).

In the present studies, we have further examined the mechanisms of action employed by *B. fuscipennis* venom in edema formation. Specifically, this study examined the role of leucocyte (lymphocytes) in the venom-induced edema. Leucocytes have always been recognized as a relevant component of inflammatory responses, and lymphocytes in

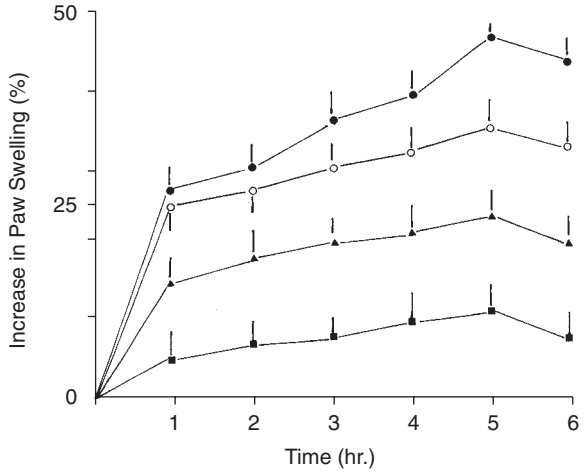

Figure 2. Time course of the edematogenic response *B. fuscipennis* venom (40 µg/paw). Data are expressed as % increase in paw swelling as compared to control paws. (●) Normal rats; (▲) Leucopenic rats; (○) Leucopenic rats injected i.v. with lymphocytes suspension; (■) Leucopenic rats injected i.v. with PMN granulocytes suspension. Each point represents mean value ± S.E.M. n = 6–10 rats.

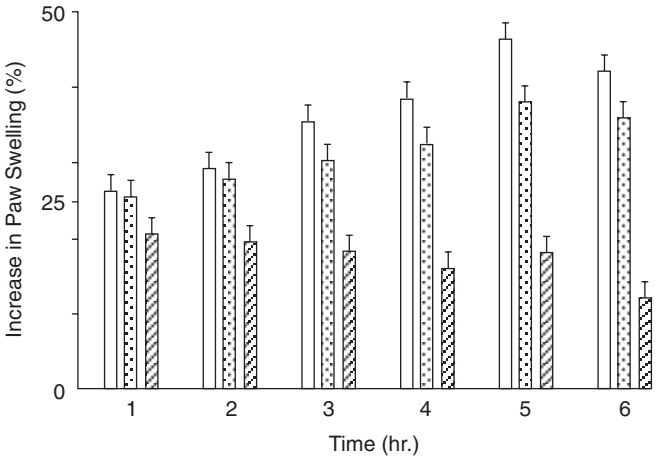

Figure 3. Time course of the edematogenic response of *B. fuscipennis* venom (40 µg/paw). Data are expressed as % increase in paw swelling as compared to control paws. Normal rats (open column); experimental obstruction of the thoracic duct (hatched column). Each point represents mean value ± S.E.M. n = 8 rats.

Table 2. Effect of thoracic duct ligation on the leucocyte counts in the rats.

Animal Groups	Total Leucocytes (per ml)	Differential Counts (Cells/ml)				
		Lymphocytes	Neutrophils	Monocytes	Eosinophils	Basophils
Normal (n = 5)						
Initial	8592 ± 385	5307 ± 265	1696 ± 344	1304 ± 132	18 ± 10	0
Final	9465 ± 392	5826 ± 311	1948 ± 297	1522 ± 152	73 ± 58	0
Sham-operated (n = 5)						
Initial	7946 ± 402	5169 ± 216	1271 ± 255	1299 ± 102	41 ± 15	0
Final	8211 ± 568	5330 ± 345	1401 ± 165	1218 ± 145	58 ± 32	0
With ligation (n = 10)	8977 ± 510	5528 ± 386	2095 ± 205	1523 ± 87	69 ± 37	0
Initial Final	5689 ± 295*	2702 ± 252*	1911 ± 103	1284 ± 91	47 ± 28	0

Leucocyte counts were performed before (initial) and 3 days after (final) the experimental obstruction of the thoracic duct. The same time intervals were observed between initial and final counts for matching groups of sham-operated and normal animals. Results are the mean values ± S.E.M. *P < 0.05 vs. initial counts.

particular are thought to play a role in the development of acute inflammatory reaction (Garcia Leme, 1981). The study of venom-induced edema can be hampered by the appearance of haemorrhage, if the venom contained potent haemorrhagic principle(s) (Bjarnanson & Fox, 1989; Borkow & Gutierrez, 1993). Therefore, the dose of *B. fuscipennis* crude venom selected for this study was such that neither haemorrhagic or necrotic changes were evident macroscopically. The test-dose of 40 µg/paw was adequate since it elicited submaximal response, thereby allowing the detection of both inhibition and potentiation of edema forming activity (Eno, 1997).

The evidence is convincing that the white blood cells are also involved in the *B. fuscipennis* venom-induced edema. This is suggested because amethopterin-induced leucopenic rats were less responsive to the venom than their matching controls. Also, interruption of lymph circulation by thoracic duct ligation resulted in a marked lymphocytopenia that was accompanied by a reduced capacity of the animals to react to the crude venom. An important finding that strongly implicated the lymphocytes in this reaction was the marked reversal of the inhibited edematogenic response by lymphocyte suspension administered to leucopenic rats. Neutrophil suspension was ineffective in this regard, and both suspensions contained sufficient amounts of the particular cells to apparently counteract the deficiency produced by the drug (amethopterin). Furthermore, the edema-forming activity of the venom was markedly suppressed when lymphocyte recirculation from lymph to blood was interrupted by thoracic duct ligation. This procedure produced inteference of the high turnover of the lymphocytes in circulation (Cury et al., 1994).

It has been adequately documented in the literature observations which indicate that lymphocytes have the capability of secreting pro-inflammatory factors for the development of acute, non-immune inflammation (Garcia Leme, 1981; Bechara, et al., 1976; Garcia Leme et al., 1976, 1977, 1978). Following injury, materials formed or transformed at the affected area might gain access to the lymphatic vessels and stimualte lymphoid cells either to release proinflammatory factors or to migrate to the site of injury where they would exert proinflammatory activities (Garcia Leme, 1981; Bersani-Amado & Garcia Leme, 1982).

In the present studies, the finding that the edematogenic response was reduced in leucopenic rats is not surprising. This is because amethopterin-induced leucopenia reduced the experimental edema evoked by phospholipase A2 from *Trimeresurus mucrosquamatous* venom (Wang & Teng, 1992). The response of the granulocytes inflammation appears to require the functional intergrity of the lymphocytic series (Hamood & Fondu, 1991), therefore, a complex regulated system involving haematogenous cells is likely to play a role in venom-induced lesions (Cury et al., 1994).

In conclusion, *B. fuscipennis* venom-induced rat paw edema is a multi-mediated phenomenon (Eno, 1997). Apart from the immense contribution of some pharmacologically active compounds in the edematogenic response, we suggest the involvement of the circulating lymphocytes in this reaction. This sugestion points to possible interactions between chemical mediators and the lymphoid cells during the edema formation. As a first step, separation of the venom into fractions according to mol. wt. is a likely progression in the investigation of the mechanism of action of the venom. Analysis of the individual fractions, based on the findings of the present study, would enable a more specific characterization of the venom, and possibly result in the isolation of the component(s) responsible for the edema and pain experienced as a result of *B. fuscipennis* envenomation.

Acknowledgement

The authors are grateful to Mr. D.D. Dakat, Faculty of Medicine, University of Jos, Nigeria for his technical assistance. Our thanks also goes to Prof. E.U. Usua (an entemologist), Department of Zoology, University of Calabar, Nigeria, for his useful ideas and very stimulating discussion, and to Miss Patience Jackson for typing the manuscript.

References

Bechara GH, Sudo LS, Ribeiro Dos Santos R, Garcia Leme J (1976): Modulation by lymphocytes of the vascular effects caused by inflammatory mediators and carrageenin in the rat. *Br J Exp Path 57*: 497–504.

Bersani-Amado CA, Garcia Leme J (1982): Some characteristics of the participation of lymphocytes in non-immune inflammation. *Br J Exp Path 63*: 463–471.

Bjarnason JB, Fox JW (1989): Hemorrhagic toxins from snake venoms. *J Toxic-toxin Rev 7*: 121–209.

Borkow G, Gutierrez JM (1993): Isolation and characteristic of synergistic hemorrhagins from the venom of the snake *Bothrops asper. Toxicon 31*: 1137–1150.

Chaves F, Barboza M, Gutierrez JM (1995): Pharmacological study of edema induced by venom of the snake *Bothrops asper* in mice. *Toxicon 33*: 31–39.

Cury Y, Teixeira CFP, Sudo LS (1994): Edematogenic responses induced by *Bothrops jararaca* venom in rats: Role of lymphocytes. *Toxicon 32*: 1425–1431.

Eno AE (1997): Pharmacological investigation of edema induced by venom from the wasp (*Belonogaster fuscipennis*). *Toxicon 35*: 1691–1698.

Gallin JI, Goldstein IM, Synderman RS (1988): Overview. In: Gallin JI, Goldstein IM, Snyderman RS eds., *Inflammation: Basic Principles and Clinical Correlates*, New York. Raven Press, pp. 1–3.

Garcia Leme J (1981): Role of lymphocyte in non-immune experimental inflammation. *Agents Actions 11*: 597–602.

Garcia Leme J, Bechara GH, Ribeiro Dos Santo R (1976): A proinflammatory factor in lymphocytes. Its role in the

development of acute, non immunological inflammatory reactions. *Br J Exp Path 57*: 377–386.

Garcia Leme J, Bechara GH, Sudo LS (1977): The proinflammatory function of lymphocytes in non-immune inflammation: effect of steroidal and non-steroidal anti-inflammatory agents. *Br J Exp Path 58*: 703–711.

Garcia Leme J, Ramos De Oliveira JC, Sudo LS, Verissimo De Mello SB (1978): Persistent inflammatory responses enhance the proinflammatory activity of lymphocytes. *Br J Exp Path 59*: 345–353.

Habermann E (1972): Bee and wasp venoms. *Science 177*: 314–322.

Hamood M, Fondu P (1991): In vivo regulation of granulocyte production in acute inflammation. *Cell Prolif 24*: 181–190.

Ho CL, Hwang LL (1991): Local edema induced by the black-bellied hornet venom and its components. *Toxicon 29*: 1033–1042.

Ho CL, Hwang LL, Chen CT (1993): Edema-inducing activity of a lethal protein with phospholipase A_1 activity isolated from the black-bellied hornet (*Vespa basalis*) vonom. *Toxicon 31*: 605–613.

Pick T (1984): Pharmacology of hymentoptera venom. In: Tu AT ed., *Handbook of Natural Toxins*. New York, Marcel Dejjer, Vol. 2, pp. 135–185.

Schmidt JO, Eno AE, Carpenter JM (2000): Erratum and letter to editor: Are we as toxinologists overlooking vouchers? *Toxicon 38*: 321–322.

Sudo LS, Garcia Leme J (1980): The inflammatory responses of rats depleted of lymphcytes or with an artificial obstruction of the thoracic duct. *Br J Exp Path 61*: 176–185.

Wallenstein S, Zucker CL, Fleiss JL (1980): Some statistical methods useful in circulation research. *Circulation Res 47*: 1–9.

Wang JP, Teng CM (1992): Role of PMN leucocytes, platelets and some mediators in the rat hind-paw oedema induced by two phospholipase A_2 enzymes from *Trimerusurus mucorsquamatus* venom. *J Pharm Pharmacol 44*: 300–305.