the fungi grow well, but their growth is of a colonial form; that is, the colonies are made up of tightly compacted hyphae. The fungus assumes a more openly mycelial state on a nutrient-poor medium.

Ahmadjian (*1*) recognizes several stages of lichen resynthesis. The first stage is the contact between the symbionts, either through appressoria or by envelopment of algal cells by fungal hyphae. The second stage is the formation by the fungus of a tissuelike mat of cells called a pseudoparenchyma. This tissue binds the algal cells into a compact group and is the forerunner of thallus differentiation into cortical, algal, and medullary layers. We did not observe pseudoparenchyma tissue in our cultures, but some of the fungal hyphae consisted of a series of short cells which suggested the beginning of such a tissue formation.

Since the algal sheath binds the fungal hyphae during the early stages of association of the symbionts, it is conceivable that such a sheath is a means by which the symbionts recognize each other. However, there is no evidence at present to support such a possibility. Recently, it has been shown that lectins, which are plant proteins, may be a way by which symbiotic bacteria are recognized and accepted by their plant hosts (*13*). Although we know that some specificity exists between lichen symbionts, that is, one cannot randomly mix different symbionts and achieve lichen formation, we still are not clear about the limits of specificity. We do not know how many different types of algal symbionts one lichen fungus can associate with and vice versa. Moreover, we are not sure of the stimulus that causes the fungus to find and make contact with the alga. There is no evidence to support a chemotropic response. Rather, the limited findings indicate that the initial response of the fungus is thigmotropic; that is, it responds to a specific surface shape. In our study, we have seen a fungal hypha form an appressorium around another hyphal cell, and in an earlier study (*7*) we observed with transmission electron microscopy the presence of intrahyphal hyphae. The algal sheath, if it does play a role in symbiont recognition, may be a secondary factor that binds the appropriate fungus and stimulates it to begin thallus differentiation.

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A Harvester Ant Venom: Chemistry and Pharmacology

Abstract. *The mammalian toxicity of the potently algogenic venom of the ant* Pogonomyrmex badius *is greater than that reported for any other insect venom. This enzyme-rich venom contains high concentrations of phospholipase A_2 and B, hyaluronidase, acid phosphatase, lipase, and esterases. This hemolytic secretion from the poison gland produces unusual symptoms in mammals and appears to have been evolved as a deterrent for vertebrate predators.*

Stings by harvester ants of the genus *Pogonomyrmex* have long been recognized for their painfulness and for the highly unusual symptoms that follow envenomation (*1*). In addition to the typical symptoms—pain, redness, burning and itching, and slight swelling—which ensue after stings by most ants, bees, or wasps, the symptoms resulting from envenomation by *P. badius* include localized piloerection and sweating plus long-lasting pain and tenderness of the nearest lymph nodes. We report our analysis of some toxicological, pharmacological, and biochemical properties of this venom.

Fresh venom from worker ants of *P. badius* was used in all experiments. In the ant, the venom is stored in a spherical reservoir (about 0.5 mm in diameter) located near the tip of the abdomen (Fig. 1). To obtain pure venom, reservoirs were removed, rinsed twice with distilled water to remove hemolymph, fat body, or other contaminants, and ruptured in a droplet of distilled water. The empty reservoirs were discarded.

The toxicity of the venom to mice was 0.42 mg/kg [LD_{50} (lethal dose for 50 percent of the animals), administered intraperitoneally] with 95 percent confidence intervals of 0.31 to 0.58 mg/kg (*2*). This is not only the highest toxicity reported from an insect venom, but also is roughly five times higher than the toxicity (LD_{50}, administered intravenously to mice) reported for the venom of the Oriental hornet and eight to ten times higher than reported for honeybee venom (LD_{50}, administered intravenously to mice) (*3*, *4*). These two venoms are the most toxic venoms reported from insects outside the genus *Pogonomyrmex*.

Venom from *P. badius* is not especially toxic to insects, nor does it produce permanent paralysis of envenomated insects. The toxicity (LD_{50}) of the venom injected into the hemocoel of larvae of the flesh fly, *Sarcophaga bullata*, or wax moth, *Galleria mellonella*, falls in the range of 50 to 100 mg/kg (*5*). Thus, the toxicity of harvester ant venom appears to be different from that of the venoms of paralyzing wasps, which are usually not lethal to the injected animal, but which may produce total permanent paralysis when as little venom as 10 to 100 μg/kg are injected (*6*).

A powerful, direct hemolytic factor is present in *P. badius* venom. As little venom as 4 μg/ml induces more than 95 percent hemolysis of a 5 percent suspension of washed mouse blood (*7*). The direct hemolytic activity is not reduced by incubation of the venom with a 100-fold excess of heparin. In this respect, *P. badius* venom is different from the venoms of the honeybee or the Oriental hornet, both of which contain direct hemolysins that are inhibited by heparin (*4*, *8*). Heparin inhibits the hemolytic activities of these two as well as other venoms through formation of acid-base complexes with venom peptides (*9*). These complexes form white precipitation zones when 100 μg of honeybee venom and 10 μg of heparin are allowed to diffuse toward each other on Ouchterlony diffusion plates (*10*, *11*). No zones of precipitation are discernible when 500 μg of *P. badius* venom are diffused toward either 10 or 100 μg of heparin. Even 100

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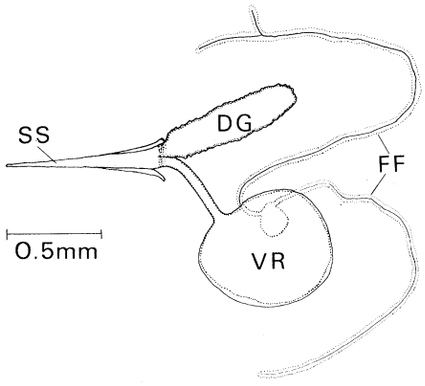

Fig. 1. Venom apparatus of the harvester ant *P. badius*. Abbreviations: *DG*, Dufour's gland; *FF*, free filaments; *SS*, sting shaft; *VR*, venom reservoir.

times as much heparin as venom does not protect envenomated mice from the lethal effects of the venom. On the basis of this evidence plus the lack of effect of heparin on the hemolytic properties of venom, it becomes apparent that heparin lacks any ability to form complexes with or reduce the activity of harvester ant venom.

Analysis of this arthropod venom revealed a number of different enzymes. Like other venoms, including those of ants, *P. badius* venom contains hyaluronidase, an enzyme that promotes the internal spreading of venom components. The activity of the enzyme in *P. badius* venom is more than 7400 NF (*National Formulary*) units per milligram (*12*). Differences in enzyme assay procedures and substrates preclude precise comparison of this value with those reported for other venoms. Nevertheless, we believe that this venom, which is about 2.5 times more active than honeybee venom in our assay (*13*), is the richest reported insect venom source of hyaluronidase.

The activities of phospholipase A_2 and phospholipase B are also elevated in *P. badius* venom. When the venom is assayed by the radial diffusion technique, phospholipase A_2 activity is about 95 IU/mg, which is greater than that of other known arthropod venoms (*14*). Phospholipase B, which hydrolyzes lysolecithins, is present in high, although nonquantified concentrations (*15*). Since this enzyme in *P. badius* venom is thermally denatured at 75° to 80°C and since phospholipase A_2 is not denatured when heated to 100°C, the two enzymes appear to be different proteins. This situation is similar to that reported for mammalian tissue phospholipases and contrasts to that demonstrated for the phospholipases in several snake venoms (*16*).

Although phospholipases were once believed to contribute little to the toxicity of venoms, this view is no longer held. The lysophosphatidyl compounds released by the action of phospholipase A_2 are capable of lysing both red blood cells and mast cells. When the latter are lysed, 5-hydroxytryptamine, histamine, and SRS-A (a slow reacting substance of anaphylaxis) are released. These compounds cause pain and delayed effects possibly due to SRS-A. Moreover, the enzymes themselves are sometimes highly toxic (*17*).

Lipase, which heretofore has not been reported in any insect or animal venom, is present in harvester ant venom at 600 units per milligram as determined by titration of free fatty acid liberated by the action of venom on an olive oil–water emulsion (*18*). This level of activity surpasses by several hundredfold the levels that could be due to contamination by hemolymph or tissues.

Acid phosphatase and esterases constitute two additional groups of enzymes present in harvester ant venom. The former, analyzed by the spectrophotometric analysis of the breakdown of *p*-nitrophenyl phosphate, exhibits an activity greater than 8 units per milligram of venom (*19*). At least three esterases are observed on polyacrylamide gels stained for esterase with α- or β-naphthyl acetate as substrates (Fig. 2). Although acid phosphatase is present in venoms from the honeybee, yellow jacket, paper wasp, and bumblebee (*20, 21*) and esterases are found in the venom of the honeybee (*20*), these enzymes apparently have not been observed previously in an ant venom.

Hyaluronidase, phospholipase, and acid phosphatase constitute the major allergens of honeybee venom (*22, 23*). Although yellow jacket, paper wasp, and harvester ant venoms contain these hydrolase activities, patients allergic to honeybee venom are not usually allergic to yellow jacket venom and vice versa (*24*). Furthermore, patients sensitive to harvester ant venom are generally not sensitive to the venoms of the honeybee, yellow jacket, or paper wasp (*25*). Whether or not allergy to nonapid venoms is induced by these enzymatically similar, but apparently immunogenetically different enzymes, remains to be established. If, however, these enzymes are the major allergens, we suspect that harvester ant venom, the richest known hymenopterous source of these enzymes, may pose a greater health threat with respect to anaphylaxis than is currently believed.

The results of our investigation and others (*26*) illustrate that ant venoms

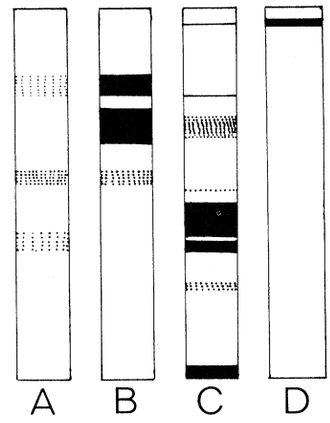

Fig. 2. Diagram of polyacrylamide gels of *P. badius* venom stained for enzymes and proteins. (A) Stain for esterase (β-naphthyl acetate substrate); (B) stain for esterase (α-naphthyl acetate substrate); (C) general protein stain (amido black); (D) stain for acid phosphatase (α-naphthyl acid phosphate substrate).

may be exceptionally toxic and contain a great diversity of pharmacologically active compounds. However, although many proteinaceous venoms of hymenopterous species contain similar kinds of hydrolases, which in certain cases are allergenically active (*22, 23*), lack of allergenic cross-reactivity between several of these venoms (*24, 25*) may indicate that the structures of these enzymes differ considerably from venom to venom. These differences are not unexpected because ants and other venomous insect groups have evolved under separate sets of environmental conditions. We believe that the algogenic venom of *P. badius* has been evolved as a deterrent for vertebrate predators (*27*). It is not unlikely that the chemistry of the venoms of other species of stinging ants will ultimately be demonstrated to be correlated with species that constitute potential predators.

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Effect of Oxygen Pressure During Culture on Survival of Mouse Thyroid Allografts

Abstract. *A marked increase in the percentage of mouse thyroids that retained function 20 days after transplantation across a major histocompatibility barrier and the percentage that lacked generalized infiltration was observed when the grafts received hyperbaric oxygen during a 4-day culture period. Perfusion of the donor animal before thyroidotomy and the addition of fetal calf serum to the culture medium did not have a significant effect on graft survival, but the percentage of grafts lacking generalized infiltration was slightly increased by the addition of hydrocortisone to the culture medium.*

Culturing an organ before it is transplanted has been reported to enhance survival of the allograft (*1*–*5*). Lafferty and co-workers described a technique for culturing mouse thyroids and grafting them under the kidney capsules of allogeneic mice (*6*–*8*). The most important aspect of this culture technique was placing the 1- to 2-mm organs on a raft at the surface of the culture medium in an atmosphere of 95 percent O_2 and 5 percent CO_2. Using the identical technique, Sollinger, *et al.* (*9*) found that rat thyroids had an enhanced survival when transplanted into mice. With both xenografts and allografts, prolonged or indefinite survival required a culture period of nearly 4 weeks.

Because of the difficulties in maintaining any organ in culture for long periods of time, we have varied conditions to find an effective culture method that requires less time. We report here the effect of perfusing the organ with culture medium before it is removed from the donor, and the effects of oxygen pressure, serum, and hydrocortisone during culture on the survival of mouse thyroid allografts.

Individual lobes of mouse thyroids were placed on microscope lens paper supported by a stainless steel wire mesh in 60-mm plastic petri dishes. Culture medium (10 ml) was added so that the lens paper was saturated and the paper was at the interface of the liquid and the gaseous atmosphere. The medium used for organ culture was Eagle's minimum essential medium (MEM) (F-15, Grand Island) supplemented with pyruvate (1 m*M*), sodium bicarbonate (2.2 g/liter), penicillin (100 unit/ml), and streptomycin (100 μg/ml). Cultures were maintained for 4 days at 37°C in sealed pressure chambers filled with 95 percent O_2 and 5 percent CO_2. Hydrocortisone acetate (10 mg/ml) was added to some cultures by diluting sterile hydrocortisone acetate suspension (50 mg/ml, Invernex) in culture medium. Before dilution, this preparation contained sodium carboxymethylcellulose (0.03 percent), polysorbate (0.026 percent), and benzyl alcohol (0.09 percent). To other cultures, fetal calf serum (FCS) (10 percent by volume) was added.

Individual BALB/c mouse thyroid lobes were placed under the kidney capsules of thyroidotomized C57BL/6 ($H\text{-}2_b$) or BALB/c ($H\text{-}2_d$) recipients. Thyroidotomy of the recipient was performed at the time of transplantation with the aid of a dissecting microscope. Particular care was taken to avoid damage to the recurrent laryngeal nerve that is located close to the left thyroid lobe. Total thyroidectomy of the recipient was not achieved.

In some cases, the donor was perfused with MEM before organ removal. The donor was anesthetized with sodium seconal, and the heart was approached through the abdomen by cutting the diaphragm. An injection of 6 ml of MEM was given in the left ventricle through a 25-gauge needle. Immediately after beginning the perfusion, the right ventricle

Table 1. Effect of perfusion before culture, and oxygen pressure, serum, and hydrocortisone acetate during a 4-day culture on ^{125}I uptake and histology of BALB/c thyroids after 19 to 21 days in a C57BL/6 host.

Group	pO_2 (mm)	Donor perfused	FCS	HC*	^{125}I† uptake	Histology‡				S + F (%)
						S	F	G	D	
1	600	+	−	−	0/11	0	0	0	11	0
2	1000	+	−	−	4/11	1	1	2	7	18
3	1300	+	−	−	8/13	4	3	1	5	53
4	1300	−	−	+	15/16	5	8	2	1	81§
5	1300	+	+	−	6/9	0	3	3	3	33
6	1300	−	−	−	10/15	2	4	4	5	40

*Hydrocortisone acetate, 10 μg/ml. †The numerator is the number of recipient mice with ten times or more ^{125}I counts in the grafted kidney than the control kidney. The lowest ratio found in ten syngeneic grafts was 12. ‡S, indistinguishable from syngeneic grafts, no infiltration; F, focal infiltration; G, generalized infiltration; D, destroyed, no visible follicles. §Significantly different from group 6, $P = .05$.

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