Disc Electrophoresis of Hymenoptera Venoms and Body Proteins

Abstract. *The venom proteins of honey bee,* Polistes *wasp, yellow hornet, and yellow jacket are similar but not identical. Extracts of venom sacs and whole insects contain several proteins not found in the pure venoms.*

The high incidence (*1*) of death from wasp, hornet, or bee sting (*2*) is generally attributed to anaphylactic shock resulting from hypersensitivity to venom proteins. Desensitization treatments consist of injecting either an extract of whole insect bodies (*3*) or of the contents of extirpated venom sacs (*4*). The recent development of methods for obtaining natural venom samples (*5, 6*) has suggested a third alternative, desensitization by injection of dilute solutions of venom. The last alternative might avoid problems resulting from injection of substances in whole-body or sac extracts not required for formation of antibodies to venom antigens and of possible hazard to the patient (*4, 7*).

In desensitization of persons hypersensitive to Hymenoptera venom it is often difficult to ascertain the identity of the stinging insect responsible for the victim's condition. Foubert and Stier have suggested the use of mixed insect extracts to insure proper desensitization (*3*), whereas Loveless has suggested that the contents of the venom sacs of *Polistes* wasps may be used to afford satisfactory protection against the stings of other Hymenoptera (*8*).

The technique of disc electrophoresis (*9*), because of its high degree of resolution and reproducibility, was selected for the study of the proteins in certain venoms, venom sac extracts, and whole-insect extracts.

Venom samples were obtained from honeybees [*Apis mellifera*], wasps [*Polistes apachus* (Sauss.)], yellow hornets [*Vespula (Dolichovespula) arenaria*], and yellow jackets [*Vespula (Vespula) pennsylvanica* (Sauss.)] by the method described earlier (*6*). Bumblebees could only rarely be induced to sting by this method, and not enough of their venom could be collected for study. Venom sac and whole-insect extracts were prepared from the aforementioned insects and also from bumblebees [*Bombus huntii* (*Gr.*) and *Bombus occidentalis* (Gr.)]. Polyacrylamide gels were prepared by the method of Ornstein and Davies (*9*). To 1-mg samples of each dried venom were added 50 μl of phosphate buffer (0.036 percent KH_2PO_4; 0.14 percent $Na_2HPO_4 \cdot 12\ H_2O$; 0.5 percent NaCl) and 100 μl of polymer-forming concentrate (3.3 percent acrylamide). The resulting solutions were added to prepared tubes of the polyacrylamide gel; the mixtures were then kept in the light for 20 minutes to bring about polymerization of the gel. For experiments on venom sacs, four sacs from each kind of insect except bumblebee (from which two sacs were taken) were extirpated under aseptic conditions, dried over phosphorus pentoxide in a vacuum at 4°C, crushed, and triturated with 75 μl of the phosphate buffer and 100 μl of polymer-forming concentrate. Filtered extract (150 μl) was added to each gel for photopolymerization. Samples of whole-insect extracts (2.0 ml)—from female insects, method of Foubert and Stier (*3*)—were lyophilized, and each residue was triturated with 150 μl of water that had been distilled three times. A 50-μl portion of each suspension was mixed with 100 μl of polymer-forming concentrate and treated as already mentioned. The best separations were obtained with a buffer mixture in the reservoir consisting of glycine and 2-amino-2-(hydroxymethyl)-1,3-propanediol (Eastman 4833, "tris") of *p*H 8.3 and ionic strength 0.042, a potential of 250 volts, and an average current of 4 ma per tube. Tubes could be capped with cellulose membranes to reduce diffusion effects (*10*). The apparatus was essentially that of Ornstein and Davies (*9*), except for the difference in power supply. Bromphenol blue was added to the upper buffer reservoir to serve as an indicator of the speed of electrophoresis. Gels were stained after each experiment with amidoschwartz 10B, and unbound dye was removed electrophoretically with 7 percent acetic acid as solvent. The migration distances of bromphenol blue bands in gels alone and gels containing samples were measured before staining to serve as references for protein migration. The R_B values were calculated for each stained band by dividing the migration distance of each band by the migration distance for the bromphenol blue band for that experiment. These R_B values were found to be much more reproducible (precision about ± 3 percent) than were calculated "mobilities."

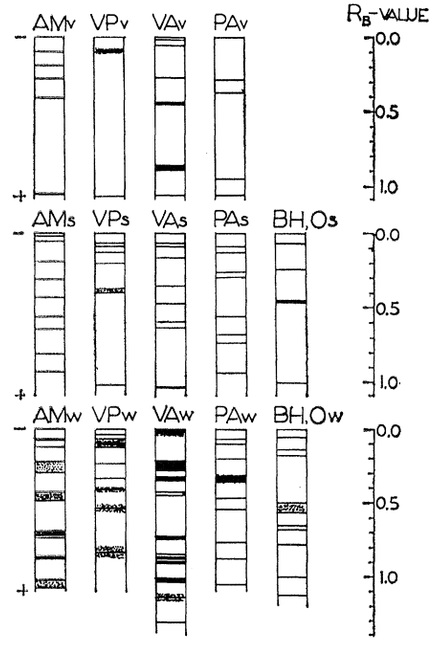

Fig. 1. Disc electrophoresis patterns of venoms, venom sac extracts, and whole-insect extracts. (*v*) Venom; (*s*) sac extract; (*w*) whole-insect extract; (*AM*) *Apis mellifera;* (*VP*) *Vespula pennsylvanica;* (*VA*) *Vespula arenaria;* (*PA*) *Polistes apachus;* (*BH, O*) *mixed Bombus huntii* and *Bombus occidentalis.*

Solutions of venom from all insects tested contained fewer proteins than did extracts of venom sacs (Fig. 1), and extracts of whole insects contained large numbers of different proteins. Differences in proteins of venom and venom sacs have been reported for the mud-dauber wasp (*Sceliphron caementarium*) (*11*). Many of the proteins in the whole-insect extracts migrated as colored bands, some because of formation of a complex with bromphenol blue and some by apparent association with insect pigments. This phenomenon has been noted with insect hemolymph samples (*12*), and the resulting R_B values are undoubtedly not characteristic of the unbound proteins. There was no appreciable association of the proteins of either the venom or venom sac with the bromphenol blue.

The electrophoretic patterns reveal one similar protein with an R_B value of about 1.07 in all the venoms studied. Certain similarities can be seen in the bands having an R_B value of about 0.09, from venoms of honeybee, yellow jacket, and yellow hornet, and in the bands having an R_B value of 0.29 and about 0.4, in venoms of honeybee, yellow hornet, and wasp. Thus, the protein compositions of the various venoms

are not identical and, if all the bands represent potentially dangerous antigens, then mixed antigens for desensitization treatment are probably needed when sensitizing insects are not definitely known. Variation in migration rates due to the more complex mixtures of proteins in sac and whole-insect extracts make species comparisons difficult with these patterns.

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Skin Tumorigenesis by 7,12-Dimethylbenz(a)anthracene: Inhibition by Actinomycin D

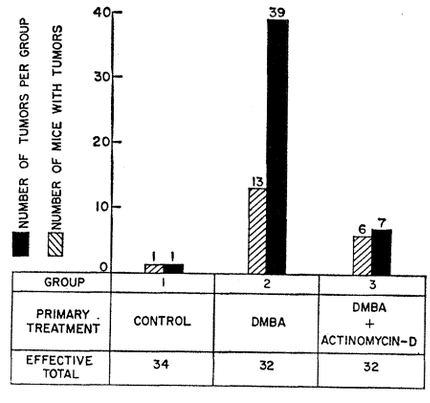
Abstract. *Topical application of actinomycin D during a short period of time immediately before and after a single application of 7, 12-dimethylbenz*(a)*anthracene markedly inhibits subsequent tumor formation. The results indicate that the initial events of carcinogenesis are dependent on DNA-dependent RNA synthesis.*

Experimental clarification of the initial changes during chemical carcinogenesis presents numerous problems. A major problem is the relatively long interval between exposure to a carcinogen and appearance of the tumors. Others are largely due to the variability in systemic transport, distribution, and metabolism of the "active" carcinogen. A system uniquely suited to the study of relevant early events in carcinogenesis is the two-stage system of skin tumorigenesis described by Mottram (*1*) and Berenblum and Shubik (*2*). In this system, a single minimal dose of carcinogen, topically applied to mouse skin, "initiates" the tumorigenic process. The "initiation" is essentially irreversible (*2*). The "initiated" centers in the skin are then "promoted" to the visible papilloma stage by repeated application of a "promoting" agent, such as croton oil, which alone is noncarcinogenic or weakly carcinogenic. Since most of the carcinogen administered by a single low dose disappears rapidly from the skin (*3*), the system allows for confinement of biochemical studies of carcinogen-induced alterations to a short time period. Also, the carcinogen is applied directly to the susceptible tissue and problems of variable transport, metabolism, and distribution of systemically administered carcinogens are minimized. Finally, the susceptible tissue is observable grossly and continuously and can be treated directly with agents of known biological activity.

In this study, we examined the effect of actinomycin D, an inhibitor of DNA-dependent RNA synthesis (*4*), on "initiation" of skin tumorigenesis by 7,12-dimethylbenz(*a*)anthracene (DMBA). Experimental procedures and results are shown in Fig. 1. Groups of 35 mice were treated once with 12 μg of DMBA alone or with DMBA and actinomycin D. Control groups received acetone in place of DMBA. One week after the treatments and weekly thereafter the mice were treated with croton oil. The animals were examined weekly for 14 weeks for the presence of skin tumors. The results show a marked inhibitory effect of actinomycin D on skin tumorigenesis. When actinomycin D was applied six times during a 20-hour period before and after treatment with DMBA (group 3), the total number of tumors was decreased by 82 percent as compared with the controls treated with DMBA (group 2). The control group given acetone only (group 1) had one mouse with one tumor, and the other control (not shown in Fig. 1) given acetone with actinomycin D (group 4) had none.

The reason for the incompleteness of the inhibition with actinomycin D is not known. We do not know the degree of effectiveness or duration of drug action of this agent in blocking RNA and protein synthesis in the skin. It is possible that adjustment of dosage and time of administration of carcinogen or inhibitor may yield complete inhibition.

No grossly visible skin damage was observed in any of the groups during the 1st week of the experiment. At 2 weeks the groups that received actinomycin D (groups 3 and 4) followed by croton oil showed visible skin damage; at 3 weeks the skin damage was less apparent; at 4 to 5 weeks it was healed. Studies designed to detect



GROUP	1	2	3
PRIMARY TREATMENT	CONTROL	DMBA	DMBA + ACTINOMYCIN-D
EFFECTIVE TOTAL	34	32	32

Fig. 1. Effect of actinomycin D on skin tumorigenesis. Four groups of 35 male Swiss mice were treated as follows: The hair was removed from the back of each mouse with electric clippers one day prior to the start of the experiment. A single dose of 12 μg of DMBA in 0.2 ml of acetone was applied topically to the entire back of each mouse in groups 2 and 3. Acetone only was applied to the mice in group 1. In group 3, 14 μg of actinomycin D in 0.2 ml of acetone was applied at 2, 5, and 8 hours before and 2, 5, and 8 hours after the application of DMBA. Groups 1 and 2 were given acetone according to the schedule of group 3. Group 4, not shown, received actinomycin D with no DMBA. One week after the treatments and weekly thereafter, all the mice received topical applications of 0.2 ml of 1 percent croton oil in acetone. The tumor yield shown is that at 14 weeks and represents only tumors persisting for a minimum of 2 weeks. Essentially the same tumor yields were noted at 12 and 13 weeks. The effective totals represent the number of survivors at the time the first tumor was observed. Control group 4, which received no DMBA, had an effective total of 27 with no tumors in the group.