PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 147 585-588 (1974)

Immune and Nonimmune Gel Precipitates Produced by Honey Bee Venom and its Components (38393)

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Investigations of bee venom (1, 2) have indicated that nonimmune gel precipitation can be obtained by diffusing bee venom or its components against normal serum. The nature of this interaction is somewhat confused since one investigator (1) suggested that this resulted from the interaction of the phospholipase A component (phos. A) with serum lipoprotein, while a second study (2) indicated that it was the mellitin component. As a result of our studies on bee venom and its components (Franklin and Baer, to be published), we have reinvestigated this phenomenon, including the "halo effect" (3), using purified components of bee venom and serum to determine more precisely the nature of this interaction.

Materials and Methods. The honey bee venoms used were Sigma Chemical Co., St. Louis, MO, lots 69B1210 (Sig. 69), 89B0080 (Sig. 89); Bureau of Biologics Reference honey bee venom (BB Ref.); Dr. H. Brown (Brown); Nutritional Biochemical Corp., Cleveland, OH, lot 4792 (NBC pure), lot 2270 (NBC Tech); and Fluka AG Chemical Co., Buchs, Switzerland (Fluka). Venoms were separated into their components by gel filtration, using Sephadex G-100; the components corresponded to those described earlier by Haberman (4). Serum from normal animals (Laboratory Aids Branch, NIH) was used, as well as normal human sera and human cord sera; the latter were obtained from Dr. H. Brown, George Washington University, Washington, DC. Cohn fractions II (globulins) and V (albumin) from human sera were obtained from the American Red Cross National Fractionation Center, Bethesda, MD. Cohn fraction II was prepared at 10 mg/ml and 30 mg/ml, and Cohn fraction V at 30 mg/ml in 0.25% saline. Alpha, β, and pre β lipoproteins, and "bottom serum" (all serum components following removal of lipoproteins) were obtained from Dr. P. Herbert, Molecular Disease Branch, NHLI, NIH. The lipoprotein fractions were isolated from pooled, normal human sera by density ultracentrifugation (5).

An antiserum against whole honey bee venom was prepared by immunization of a rabbit with Sig. 69 bee venom in complete Freund's adjuvant. Double diffusion in gel was performed by cutting wells in coated microscope slides, or by the use of plastic templates placed on the slides, as described by Crowle (6). The microscope slides were coated with 3 ml of either 1% agar or ½% agarose in 1% sodium azide. After 24–48 hr of diffusion, all gels were photographed and then stained with either 1% amidoschwarz for protein, or oil red O for lipid (7).

Results. When bee venom was diffused against normal sera from humans (adult and cord), goats, sheep, rabbits, rats, and horses, a single or occasionally double precipitin band formed in the gel. Six different bee venoms, each at 5 mg/ml, demonstrated such precipitates when diffused against normal rabbit serum, as well as rabbit immune serum (Fig. 1). The intensity of the nonimmune band varied for each venom, and for the same venom against different sera. The absence of one of the immune lines for two bee venoms reflected the absence of hyaluronidase (hyal.) for these venoms. When this gel was stained for lipid (Fig. 1, bottom), only the area corresponding to the nonimmune precipitate stained for both immune and nonimmune sera. In addition, a precipitate formed

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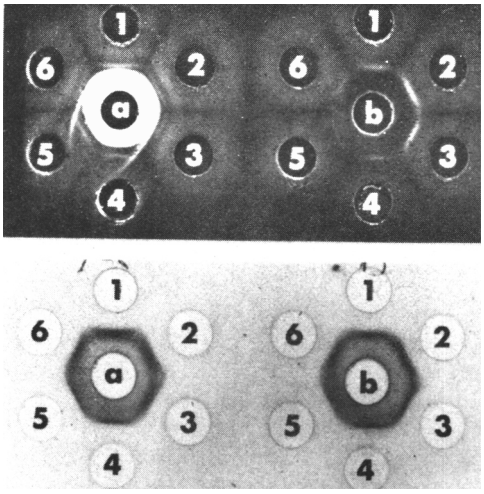

FIG. 1. Agar gel diffusion pattern from six different honey bee venoms against rabbit anti-whole bee venom (Sig. 69) serum (a) and against normal rabbit serum (b). Top-unstained, bottom-same gel stained for lipid. All six venoms demonstrate lipid-containing, nonimmune precipitin bands. 1-Sig. 69; 2-BB Ref.; 3-Brown; 4-NBC Tech; 5-Fluka; S-Sig. 89.

around wells containing venom, which did not stain with the lipid stain. In no instance could lines be observed when venom was diffused against Cohn fractions II and V at normal serum concentrations.

When several purified bee venom components were diffused against sera (Fig. 2), a lipid-containing precipitate occurred only with whole venom and mellitin. Phos. A and hyal. failed to show a line at concentrations equivalent to those found in whole venom. Additionally, a precipitate formed in the gel surrounding the wells containing whole venom and mellitin.

The three lipoprotein fractions consistently yielded heavy gel precipitates which could all yield lines of identity with one another (Fig. 3). Although whole sera frequently demonstrated two lines with venom, the lipoproteins showed only single lines. This double line was seen when venom was placed in the large center well, and never vice versa. No lines were seen when bee venom was diffused against bottom serum.

The "halo" effect surrounding wells in agar containing venom was evident whether serum did nor did not diffuse from the center well, but in no instance was a precipitate seen around wells in agarose gel (Fig. 4). However, an intense precipitin band was still seen in agarose between sera and venoms or mellitin (Fig. 4).

Discussion. When gel diffusion studies are carried out using antisera and antigens, it is usually assumed that the precipitates are composed of antigen–antibody complexes. In this study we have demonstrated that both immune and nonimmune precipitates may occur simultaneously. This is seen in Figs. 1 and 2. Unless these two types are clearly separated, there can be considerable confusion in interpretation of the results of such tests. In this instance, mellitin, the substance in bee venom that gives the nonimmune precipitate, is probably not antigenic. Attempts to produce an antibody to mellitin have yielded no success, and only the nonimmune precipitate is formed between this substance and serum.

Evidence has been presented for a lipoprotein–mellitin interaction to explain the nonimmune precipitate formed in gels between honey bee venom and normal sera. The facts which bear on this conclusion are: (a) all unfractionated

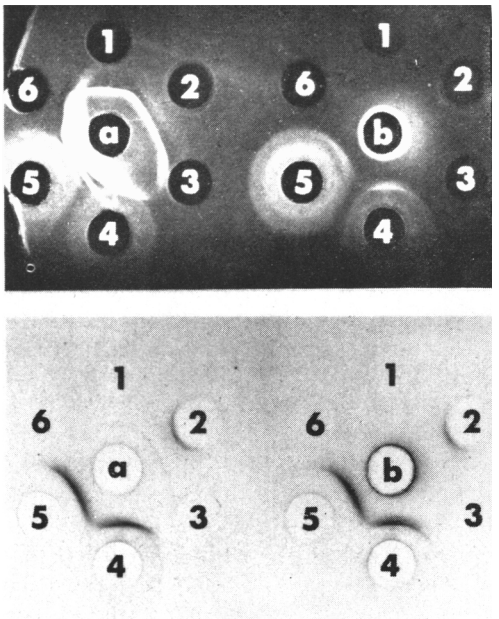

FIG. 2. Agar gel diffusion pattern for bee venom and its components against rabbit anti-whole bee venom serum (a) and against normal rabbit serum (b). Top-unstained, bottom-same gel stained for lipid. Well 5, top, demonstrates the variability in intensity of the nonimmune line with the two different sera, a and b. Faint line between 1 and a, and 2 and a, bottom, did not stain red. 1-Phos. A; 2-Sig. 69; 3-saline; 4-mellitin, 3 mg/ml; 5-Sig. 69, 5 mg/ml; 6-Hyal.

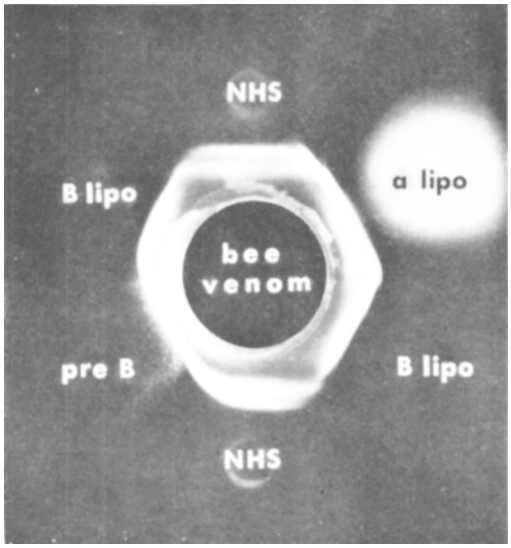

FIG. 3. Agar gel diffusion with Crowle technique between Sig. 69 bee venom, 1 mg/ml, and normal human serum (NHS) or various lipoproteins (α lipo = α lipoprotein; β lipo = β lipoprotein; pre-β = pre-β lipoprotein). Precipitin bands appear to form lines of identity between dissimilar lipoproteins. NHS demonstrates two lines by this technique.

normal animal sera yielded a precipitin line with bee venom; (b) human cord sera, Cohn fractions II and V did not produce a precipitin line; thus, neither globulins nor albumin participate in this interaction; (c) a serum lipid component was demonstrated to participate in the formation of the line since it stained with oil red O and failed to appear using bottom serum, a material free of lipoproteins; (d) purified lipoproteins were shown to complex with bee venom; (e) the mellitin fraction from bee venom and not phos. A nor hyal. produced the precipitate with normal sera.

This conclusion confirms the speculations of others (2) who, in addition, described a similar gel precipitin line between other hymenoptera venoms and normal rabbit sera. The nature of this interaction may be explained by the complexing of highly charged mellitin molecules with lipoproteins. Such a mechanism was demonstrated for the complexing of heparin with charged snake venom components (8). Since highly purified phos. A was shown not to participate in the formation of the gel precipitate, it seemed unlikely that serum lipoprotein served merely as substrate for an enzyme reaction (3).

A charge interaction probably produced the "halo" effect seen in agar about wells filled with bee venom or mellitin. When agarose was employed in place of agar, this concentric precipitate did not appear. Agarose differs from agar by being less acidic. Since mellitin is a basic protein (4), the precipitate about wells in agar may have resulted from charge interactions, which then lead to complex formation and precipitation.

Nonimmune complexing of macromolecules has been observed in many systems, and the

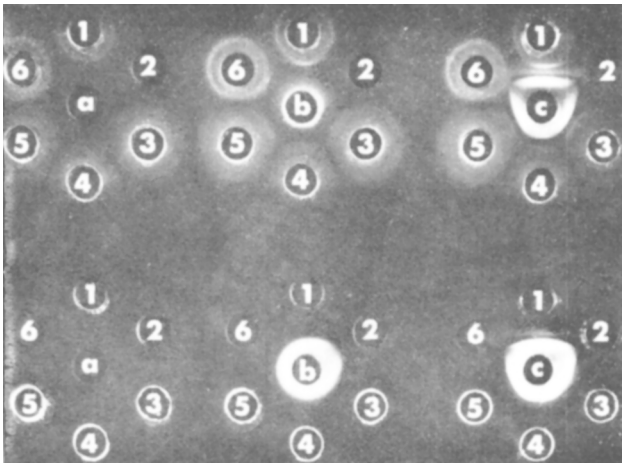

FIG. 4. Agar gel diffusion (top) and agarose gel diffusion (bottom) between four different bee venoms, 5 mg/ml, or mellitin, 3 mg/ml and: (a) no serum in center well, (b) normal rabbit serum in center well, (c) rabbit anti-whole bee venom serum in center well. The "halo" effect about outer wells occurred only with agar and appeared independently of the contents of center wells. Mellitin formed an intense nonimmune precipitate in agarose (b, bottom). 1-Sig. 69; 2-saline; 3-NBC pure; 4-NBC tech; 5-Fluka; 6-Mellitin, 3 mg/ml.

present investigation defined yet another instance. Although the physiological consequences of this interaction between mellitin and lipoproteins have not been determined, an awareness of this interaction during immunochemical investigations of bee venoms is critical.

Summary. Nonimmune complexing of macromolecules has been observed in many systems, and in studies with honey bee venom such a phenomenon was suspected. The present study attempted to define the components of an interaction observed during gel diffusion between venom and serum.

Various honey bee venoms and individual components of venom were diffused in agar or agarose against sera from numerous animal species and serum components. Whole venom or mellitin yielded precipitin bands with whole serum or lipoprotein components. Other venom and serum components failed to yield a precipitin line. In addition, a "halo" effect was noticed around whole venom or mellitin in agar, but not agarose.

A possible basis for these nonimmune interactions may relate to the highly charged nature of the mellitin molecule. An awareness of these interactions would seem critical in any immunochemical investigations of honey bee venom.

This study establishes that both immune and nonimmune precipitates may occur simultaneously in gel-diffusion studies. Unless care is taken, the interpretation of such tests can result in erroneous conclusions concerning the antigenicity of certain substances.

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Received March 28, 1974. P.S.E.B.M., 1974, Vol. 147.