Reports

Isolation of a Hemolytic Component of Fire Ant Venom

Abstract. A crystalline hemolytic principle, shown to be a constituent of fire ant venom and having the properties of an amine, was isolated from crude extracts of whole ants. The chromatographic procedure of isolation is described, and a preliminary report is given about some properties of the substance.

Interest in the responses to the sting of the fire ant (*Solenopsis saevissima*) and in its venom has been indicated in several recent publications (*1–3*). It has been reported that human subjects stung by fire ants show reactions of two types: a local necrosis and a systemic reaction presumably due to allergy (*1*). The present report (*4*) represents the first stage of our investigations of the nature of the necrotoxic principle(s) of the venom.

In studies (*3*) of some of the pharmacological properties of crude methanol-chloroform extracts of ants and of pure venom collected from the tip of the stinger, it was found that both preparations exhibit strong hemolytic activity. That this activity is confined to the venom was indicated by the following experiment: An extract prepared from 95 ants whose venom sacs had been carefully dissected out was found to be virtually devoid of activity, while the contents of a single sac was highly hemolytic.

The hemolytic principle appeared to be nonprotein in nature because crude extracts showed no sensible loss of activity on exposure to heat (100°C, 1 hr), the active component of extracts was soluble in a nonpolar solvent such as petroleum ether, and pure venom was Ninhydrin-negative. More insight into the nature of the hemolytic principle was gained from the observation that pure venom is alkaline in reaction and gives a positive nitrogen test, and that preparations lose their activity upon exposure to cation exchangers. These findings indicated that the hemolytic principle was associated with the alkaline constituents of the venom and was probably an amine.

These observations suggested a sensitive method of assay for at least one component of venom, the hemolytic principle, and led us to concentrate our efforts on isolating this component from crude extracts. This appeared to be preferable to our earlier plan of fractionating pure venom, the collection of which required enormous time and labor.

The method used to measure hemolytic activity is a modification of the procedure described by Wilbur and Collier (*5*). An aliquot of the material to be tested is taken up in 0.2 ml of ethanol and mixed with 3 ml of a stock suspension of washed rabbit erythrocytes in isotonic buffered saline (4.53 g of NaCl per liter of 0.067*M* phosphate buffer, *p*H 7.0). Hemolysis results in a decrease of the opacity of the suspension; this in turn is reflected in a decrease in optical density when readings are made at λ 660 mμ in a spectrophotometer. The more potent the preparation, the less time it requires to bring about hemolysis.

To isolate the hemolytic component, ants are homogenized in tissue grinders, 20 ml of methanol being used per gram of ants, and the clear extract is separated. The residue is reextracted in a similar manner with chloroform. The combined extracts are evaporated to a thick, oily mass in a flash evaporator, at 40°C. The concentrate is taken up in petroleum ether, from which medium the active material is adsorbed on silicic acid (Mallinckrodt, A. R., 100 mesh). This is then eluted with a 2:3 mixture of acetone and petroleum ether, until the effluent is free of activity. The concentrate from the eluate is taken up in a neutral, 1:1 mixture of methanol and acetone. Treatment of this preparation with carboxymethylcellulose (Brown Co., CM-S, type 20) transfers the active material to the ion exchanger. The cellulose is then treated with an excess of phosphate buffer (*p*H 7.0), 0.25*M*, and the mixture is extracted with petroleum ether followed by chloroform. The concentrate from the combined extracts is dried over phosphoric anhydride under vacuum. It is then dissolved in dry ethyl ether (about 10 ml per gram of concentrate), and the solution is chilled in an ice bath. When dry HCl is bubbled through this solution, the active principle separates as a white precipitate, presumably as the hydrochloride. This is washed free of the yellow mother liquor with more ethyl ether. It is then recrystallized twice from a mixture of carbon tetrachloride and petroleum ether and once from a mixture of ethanol and ethyl ether. Starting with a 27-g batch of ants, we obtained 160 mg of crystals and some 75 mg of less pure material.

The hydrochloride melts at 144° to 145°C, and it responds to the tests for tertiary amines. It is soluble in chloroform, carbon tetrachloride, and ethanol, slightly soluble in ethyl ether, and insoluble in water and petroleum ether. The free amine appears to be soluble in the usual fat solvents and insoluble in water.

The crystalline material is potently hemolytic: addition of 100 μg to 3 ml of the stock erythrocyte suspension brought about complete hemolysis in less than 15 seconds. The hemolytic effect is slowed considerably in the presence of serum and in systems containing cholesterol.

Further studies now in progress may enable us to assess the relationship of this substance to other constituents of the venom.

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References and Notes

1. R. Caro, V. J. Derbes, R. Jung, *A. M. A. Arch. Dermatol.* 75, 475 (1957).
2. M. S. Blum, J. R. Walker, P. S. Callahan, A. F. Novak, *Science* 128, 306 (1958). This report appeared when we were in the process of studying the properties of pure venom collected by a method similar to the method described by these authors. Our findings about the venom were in agreement with theirs.
3. E. O. Wilson, *ibid.* 129, 643 (1959).
4. This work was supported by a research grant (E 1755 C-1) from the U.S. Public Health Service.
5. K. W. Wilbur and H. B. Collier, *J. Cellular Comp. Physiol.* 22, 233 (1943).

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].