6. Kleinholz, L. H., *Anat. Rec.,* 1940a, v78, Suppl., 140.

7. ———, *Biol. Bull.,* 1940b, v79, 432.

8. Drager, G. A., Blount, R. F., *Anat. Rec.,* 1941, v81, Suppl., 92.

9. Lee, T. H., Lerner, A. B., *Pigment Cell Biology,* Acad. Press, N. Y., 1959, p435.

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Further Characterization of Hemolytic Component of Fire Ant Venom, Mycological Aspects. (25351)

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Preliminary observations about the antibiotic character of venom were made by Caro *et al.*(3), who noted that of pustules produced by experimental stings, all, with one exception, were bacteriologically sterile. On one occasion non-hemolytic staphylococci were isolated, but organisms were not seen in tissue removed for biopsy. Using the paper disk method, Blum *et al.*(2) demonstrated the effectiveness of venom against *Micrococcus pyogenes, Streptococcus pyogenes, Escherichia coli, Lactobacillus casei* and a variety of molds which were not named. The present report is concerned with the antimycotic effects of crystalline, hemolytic component of fire ant venom isolated by Adrouny *et al.*(1). It is an attempt to add another means of biological characterization of the crystalline material and is not to be interpreted as a study on the antimycotic effects of this material for future clinical use.

TABLE I. Effect of the Crystalline Hemolytic Component of Fire Ant Venom on Fungal Growth.

Inhibition of growth	No inhibition of growth
Blastomyces dermatitidis	*Epidermophyton floccosum*
Candida albicans	*Microsporum Audouini*
Cladosporium Wernecki	" *canis*
Cryptococcus neoformans	*Monosporium apiospermum*
Geotrichum sp.	*Oospora* sp.
Hormodendrum compactum	*Sporotrichum Schenckii*
Hormodendrum Pedrosoi	
Microsporum gypseum	
Nocardia asteroides	
" *brasiliensis*	
Phialophora verrucosa	
Trichophyton mentagrophytes	
Trichophyton rubrum	
" *tonsurans*	
Trichosporon Beigelii	

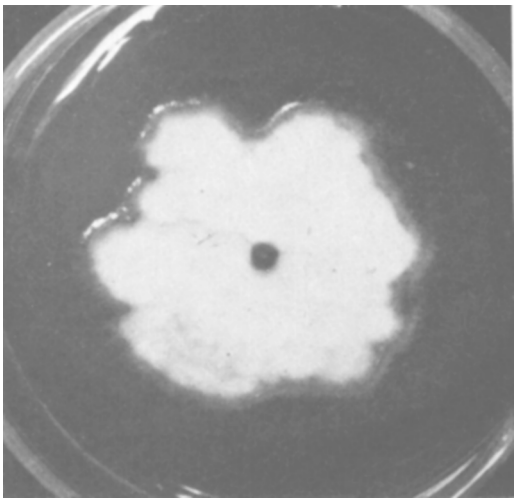

FIG. 1. Inhibition of *Trichophyton rubrum* by a crystal of hemolytic component of fire ant venom.

Methods. A series of fungi, mostly human pathogens, were streaked onto Petri dishes containing a medium consisting of 2% glucose, 1% yeast extract, 1% casamino acids and 2% agar in distilled water. All cultures were tested with a single lot of crude crystalline venom which had a melting point of 139-140°C and was insoluble in aqueous media. A few cultures were also tested with a recrystallized venom having a melting point of 144°-

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145°C similarly insoluble. A small crystal of venom was placed on each of the seeded plates which were then incubated at room temperature (25°-30°C). All crystals were approximately but not exactly the same size. The results were recorded when growth was at its maximum.

Results. Of 21 organisms tested, 15 were inhibited by the hemolytic component of fire ant venom. (Table I). Results obtained with the 2 lots of crystals were similar. An example is shown in Fig. 1.

Summary. The crystalline hemolytic component of fire ant venom was tested against 21 organisms, mostly human pathogens, by placing a crystal in direct contact with Petri dishes seeded with a different organism. Antimycotic action was demonstrated by a lack of growth immediately surrounding the crystal with the following genera: *Blastomyces, Candida, Cladosporium, Cryptococcus, Geotrichum, Hormodendrum, Microsporum, Nocardia, Phialophora,* and *Trichophyton.*

1. Adrouny, G. A., Derbes, V. J., Jung, R. C., *Science,* 1959, v130, 449.

2. Blum, M. S., Walker, J. R., Callahan, P. S., Novak, A. F., *ibid.,* 1958, v128, 3319.

3. Caro, M. R., Derbes, V. J., Jung, R. C., *A.M.A. Arch. of Derm.,* 1957, v75, 475.

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Antioxidant Effect of Chlorpromazine. (25352)

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Phenothiazine and its derivatives have been known for many years as potent antioxidants, and are used in the petroleum industry for that purpose(1). This fact suggested their trial as inhibitors of formation of lipide peroxides in biological systems. Rat brain and liver homogenates and liver mitochondria were used, and the amount of fatty acid peroxide was estimated by the thiobarbituric acid method(2). Chlorpromazine, and derivatives which are formed from it on standing in light, markedly inhibit formation of peroxides, and some properties of the reaction are described below.

Methods. Rat liver or brain was removed, iced, homogenized in 0.05 M phosphate buffer at pH 6.8, and diluted 1:10. Usually 0.4 ml of the homogenate was added to the drug solution and water to make 2 ml, and the flasks incubated aerobically at 38°. After incubation, 4 ml 20% trichloracetic acid were added, then 3 ml water, and 1 ml 0.75% thiobarbituric acid solution. The mixture was heated for 15 min in boiling water bath, centrifuged, and the red color produced by the reaction of fatty acid peroxides with thiobarbituric acid was estimated using a 520 filter. Color units are arbitrary.

Results. When solutions of chlorpromazine remained in light they rapidly darkened. These colored solutions gave no absorption peaks in the visible spectrum. The chemistry of change is not fully understood and quantitative relationship of the dark colored product to the original compound is therefore unknown. However, with the homogenized brain or liver, the antioxidant activity of the colored solution was about 5 times that of fresh material. Maximal activation occurred in a few days, and recognizable activation could also be observed in a solution kept for 24 hours at 38° in the dark. Fig. 1 shows that lipide peroxide formation in liver homogenates by both original and "activated" chlorpromazine was proportional, within limits, to their concentrations. If the concentration of chlorpromazine was kept constant and amount of liver homogenate varied, there was a linear decrease of inhibition with increasing amounts of homogenate. Thus, with 0.8, 0.4 and 0.2 ml of homogenate, 12.5 μg/ml chlorpromazine inhibited 21, 46, and 90% respectively. Percent inhibition by both chlorpromazine and its "activated" product was constant throughout the incubation period, when measured at intervals between 15 and 90 min. Thus the liver