of mestranol(12) when given by gavage and the compound is a potent antiestrogen. Besides its well recognized progestational effect, norethindrone appears to decrease the output of the luteinizing hormone (rabbit anti-ovulation test) but is less effective in blocking the release of FSH (parabiotic rat assay). In a daily dose of up to 400 μg in the immature male rat and a dose of 1000 μg in the pregnant female rat no androgenic effects were noted. These findings are in agreement with our previously published results(10).

Summary. Norethindrone (17α-ethynyl-17β-hydroxyestr-4-en-3-one) (estrogen-free) is 0.3% as active as estrone by gavage and by subcutaneous injection by a mouse uterotrophic assay. This steroid, free of estrogens, is about 3 to 5 times more active than previously studied material containing an estrogen contaminant. Both materials exhibit the same progestational activity when administered orally in the Clauberg assay and when studied in an oral rabbit anti-ovulation assay. Norethindrone (estrogen-free) has been studied in castrated female-intact female and castrated male-intract female parabiotic pairs in a rat spermatogenesis inhibition test, and in the pregnant rat.

1. Bowers, A., Villotti, R., Edwards, J. A., Denot, E., Halpern, O., J. Am. Chem. Soc., 1962, v84, 3204.
2. Rubin, B. L., Dorfman, A. S., Black, L., Dorfman, R. I., Endocrinology, 1951, v49, 429.
3. Emmens, C. W., in Methods in Hormone Research, Vol. II, p59, ed., R. I. Dorfman, Academic Press, New York, 1962.
4. Dorfman, R. I., Kincl, F. A., Ringold, H. J., Endocrinology, 1961, v68, 17.
5. ———, ibid., 1961, v68, 43.
6. Miyake, T., in Methods in Hormone Research, Vol. II, p135, ed., R. I. Dorfman, Academic Press, New York, 1962.
7. Kincl, F. A., Dorfman, R. I., Acta Endocrinol., 1963, v62, Suppl. 73.
8. Shipley, E. G., in Methods in Hormone Research, Vol. II, p179, ed., R. I. Dorfman, Academic Press, New York, 1962.
9. Kincl, F. A., Maqueo, M., Dorfman, R. I., Acta Endocrinol., 1965, in press.
10. Kincl, F. A., Dorfman, R. I., ibid., 1962, v41, 274.
11. Kincl, F. A., Endokrinologie, 1961, v40, 17.
12. Dorfman, R. I., unpublished.

Received January 5, 1965. P.S.E.B.M., 1965, v119.

Toxicity of Ant Venom, Further Studies of the Venom from *Pogonomyrmex barbatus.** (30175)

MARTIN W. WILLIAMS† AND CHARLES S. WILLIAMS
U. S. Veterans Administration Hospital, Tucson, Ariz.

McCook(1), in his excellent monograph, The Natural History of The Agricultural Ant of Texas, *Pogonomyrmex barbatus,* presents observation of the aspects of the sting of this myrmicinae which parallel closely the observations of this author(2).

These earlier studies of venom prepared from homogenates of the entire abdominal segments of the desert ant, *Pogonomyrmex barbatus, barbatus,* indicated the presence of a highly toxic cholinergic material. Blum and Ambrose(3) described a water soluble cholinergic and histamine-like venom from the army ant, *Eciton burchelli.* Venom from the fire ant, *Solenopsis soevissima,* was described by Caro *et al*(4), who also mentioned one human fatality resulting from this agent. Cavill(5) in a recent study of the venom of the Australian bull ant, *Myrmecia gulosa,* found a water soluble proteinaceous material having ultraviolet absorption peaks at 204 and 277 to 282 mμ.

The purposes of the present study were to expand upon collection procedures previously described(2) and relate the amount of venom extracted by these new techniques with the

* This project is supported in part by NIH Grants GM 11991-01 and CA 07775-01 CY.

† Research Associate, College of Pharmacy, Univ. of Arizona.

mammalian toxicity of the material. Additionally, some of the physical and chemical characteristics of the purified venom will be described.

Materials and methods. Worker ants, *Pogonomyrmex barbatus*, were collected in large numbers by placing sunken silicone treated jars in or near the openings of the formicaries. For stimulation of venom secretion, groups of 200 animals thus collected were placed in a 400 ml beaker, along each side and running to the bottom of which were 2 electrodes made of 0.040-inch platinum wire. These electrodes were attached to the secondary of a Harvard inductorium, the primary of which was coupled to groups of nickel-cadmium storage cells. Stimulation of the mass of insects was accomplished through addition of 10 ml of distilled water added in the bottom of the container.

Stimulation switching intervals were timed by means of a 110 volt AC synchronous motor and 4 cam operated micro switches which were so adjusted as to close the circuit for 26 seconds and open it for a 21-second rest period. Two such stimulus intervals with a 4.9 volt inductorium input, were followed by 2 similarly timed stimulus intervals and rest periods, with a 6.2 volt input. Thus, 2 medium and 2 higher voltage stimuli interspersed with rest periods were utilized for one cycle. Cycling was continued for 15 minutes.

The Figure illustrates schematically the apparatus utilized for stimulation. At the end of the cycling procedure the entire contents of the beaker, ants and venom-containing water were filtered through a standard conical laboratory filter paper. The animals during this time were seemingly anesthetized by the high voltage electrical shocks, and most appeared dead at the end of the run. Three milliliters of distilled water were poured over the animals and through the filter in 2 distinct 1.5 ml rinses. Within 15 to 30 minutes the animals had recovered and appeared normal in all respects.

The filtrate, a slightly opalescent solution, was centrifuged at 2000 *g* in a clinical centrifuge for 10 minutes and the supernatant clear solution was removed and lyophilized or oven dried for future tests to be described.

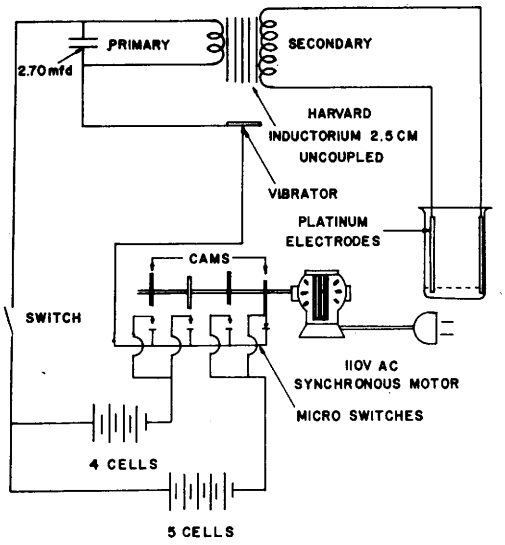

FIG. 1

Individual lots of venom were arbitrarily quantitated in terms of ant units (AU) per milliliter, *i.e.*, the number of ants from which venom was removed per milliliter of final volume.

Thirty-one 4-ml samples of the watery extract were placed in tared weighing flasks and evaporated to dryness in an oven at 58°C ± 1.5°C. Following this process the flasks were reweighed.

Ten milliliter samples of venom solution were lyophilized to a light fluffy semi-crystalline appearing material at 90°F shelf temperature and a vacuum of 0.5 mm to 5.0 μ of mercury. This material was stored and reconstituted as desired for experiments.

Thirteen 4-ml samples of filtered venom solution were prepared by filtration through 0.22 μ Millipore® filter or by centrifugation. The ultraviolet spectrum was studied by means of a Beckman DU.

Young, 2 kg mongrel dogs were injected subcutaneously and intraperitoneally with 100 ant units each of reconstituted venom and observed.

Toxicity studies were made utilizing the dosage progression of Deichmann and LeBlanc(6) in Swiss Webster male mice both prior to and after reconstitution of the evaporated venom samples.

Results and discussion. Dry weight determination of 31 4-ml samples taken from 13 separate groups of 150 or 200 ants each, resulted in a mean venom weight per sample of 0.55 mg ± 0.02 mg SE. Each ml thus contained an average of 0.14 mg of material.

The intraperitoneal injection of freshly prepared venom extract at levels of 0.56, 0.86, 1.29, 1.94 and 2.92 mg per kilo resulted in an LD_{50} of 1.29 mg per kilo ± 30%. When the same material was subjected to evaporation at 58°C ± 1.5°C to dryness, and reconstituted with distilled water, the following intraperitoneal dosage levels, 2.92, 4.44, 6.67, 10.00 and 15.00 mg/kg showed the LD_{50} to be 10.00 mg/kg ± 30%, thus, the process of evaporation and reconstitution decreased the toxicity to about one-eighth the original potency.

Deaths were preceded by a characteristic twisting and stretching of the abdomen, with dragging of the lower abdomen on the cage floor. This action was followed by respiratory distress and ataxia, then extreme lethargy with labored, slowed breathing. The final action being a burst of excitation, clonic convulsions and death apparently due to hypoxia, which occurred from 1½ to 8 hours later.

The UV spectrum showed a peak absorption in the 13 samples tested between the extremes of 265 and 285 mμ with a mean of 270 mμ. This value is rather similar to the values obtained by Cavill(5) of 277 to 282.

Lyophilized samples showed a finely crystalline-like structure and were highly water soluble. When reconstituted with distilled water and injected subcutaneously and intraperitoneally at one hundred ant units each into young dogs, a very characteristic series of reactions was noted. When given intraperitoneally the material produced retching, vomiting and diarrhea with extreme and almost continuous straining for defecation for a period of about 2 hours. The straining was interspersed with periods of apparent exhaustion. During these straining periods, the animals assumed the arched back and stance of defecation.

When given subcutaneously, no vomiting nor diarrhea was observed but the animal assumed stances indicative of extreme irritation, often somersaulting in the cage. No excessive impulse to defecate was noted after subcutaneous injection.

Vomiting in small children stung by these animals is a common experience in the Southwest and we may assume that man, being perhaps more sensitive to the venom of the ant, probably reacts differently from the dog after a sting. McCook indicates that vomiting is characteristic of a large number of stings in man(1) as well as vertigo, palpitation, dimness of vision, oppression and fainting.

Summary. As previously suggested from earlier and cruder extraction procedures(2) and as now verified, the actual venom material is far more toxic than the earlier homogenized agent. The present work indicates that 1.29 mg/kg of the relatively pure material is equivalent to 24 mg/kg of the abdominal homogenate utilized previously(2). The pure extract is capable of induction of all toxic phenomena previously described, is a protein or polypeptide, and once oven dried is not as toxic when reconstituted. The UV spectrum indicates a material with an absorption peak of about 270 mμ. The powdered crystalline material resulting from lyophilization of venom extracts is highly water soluble and may be considered as one of the more toxic of naturally occurring substances. Studies are continuing.

The authors wish to thank Dr. Floyd G. Werner, Entomology Department of University of Arizona, for help in classification of the ants utilized in this study.

1. McCook, H. C., The Natural History of the Agricultural Ant of Texas, 1879, J. B. Lippincott Press, Philadelphia, Pa.

2. Williams, M. W., Williams, C. S., Proc. Soc. Exp. Biol. and Med., 1964, v116, 161.

3. Blum, M. S., Ambrose, J. E., Entomol. Soc. Am., 9th Miami, 1961.

4. Caro, M. R., Derbes, V. J., Jung, R., Arch. Dermatol., 1957, v75, 475.

5. Cavill, G. W. K., Robertson, P. L., Whitfield, F. B., Science, 1964, v146, 79.

6. Deichmann, W. B., LeBlanc, T. J., J. Ind. Hyg., 1953, v25, 415.

Received January 12, 1965. P.S.E.B.M., 1965, v119.