(12) Dohme, A. R. L., Cox, E. H., and Miller, E., *J. Am. Chem. Soc.*, 48, 1688 (1926).

(13) Gabriel, S., *Ber.*, 15, 834 (1882).

(14) Goshorn, R. H., and Degering, Ed. F., Presented before the Medicinal Division of the American Chemical Society, Rochester Meeting (1937).

(15) Jacobs, W. A., Heidelberger, M., and Rolf, I. P., *J. Am. Chem. Soc.*, 41, 458 (1919).

(16) Johnson, T. B., and Lane, R. W., *Ibid.*, 43, 348 (1921).

(17) Klarmann, E., Gates, L. W., and Shternov, V. A., *Ibid.*, 54, 298 (1932); Klarmann, E., Gates, L. W., Shternov, V. A., and Cox, E. H., *Ibid.*, 55, 2576, 4657 (1933); Klarmann, E., Shternov, V. A., and von Wowern, J., *J. Bact.*, 17, 440 (1929).

(18) Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 39, 586 (1919).

(19) Labes, R., and Jansen, E., *Arch. exptl. Path. Pharmakol.*, 158, 1 (1930), from *C. A.*, 25, 3899 (1931).

(20) Moore, M. L., Day, A. A., and Suter, C. M., *J. Am. Chem. Soc.*, 56, 2456 (1934).

(21) Orton, K. J. P., *J. Chem. Soc.*, 79, 1354 (1901).

(22) Petrenko-Kritschenko, P., *Ber.*, 17, 2240 (1892).

(23) Robertson, G. R., "Organic Syntheses" II, 59, 60 (1922); "Organic Syntheses" III, 11 (1922).

(24) Sandemeyer, T., *Ber.*, 17, 1633 (1884).

(25) Shriner, R. L., and Fuson, R. C., "The Systematic Identification of Organic Compounds," John Wiley & Sons, Inc., N. Y. (1935).

(26) Stephen, H., Short, W. F., and Gladding, G., *J. Chem. Soc.*, 117, 510 (1920).

(27) Suter, C. M., and Hansen, H. L., *J. Am. Chem. Soc.*, 54, 4101 (1932).

INVESTIGATIONS ON THE CHEMISTRY AND PHYSIOLOGY OF THE VENOM OF THE HONEY BEE (*APIS MELLIFICA*).*

BY W. M. LAUTER AND O. J. GRIGGS.

The recent investigations of Hahn and collaborators (1, 2, 3, 4), Reinert (5) and Essex, Markowitz and Mann (6) have thrown considerable light upon the chemical and physiological properties of bee-venom. The earlier work by Flury (7), Langer (8) and Phisalix (9) described methods of obtaining the venom which soon were shown to be less efficient than those adopted by later investigators. Beck (10) gives a description of obtaining the venom for laboratory purposes which appears to be best suited to obtain as little contaminated a venom solution as is possible.

The venom is produced by the insect in the so-called poison-sac by mixing the secretions of three glands. Two of these are known as the acid glands while the third one is called the alkaline gland, because of the p_H of their secretions. Carlet's (11) experiments seemed to show that only the mixture of the two liquids has the typical toxic properties of bee-venom. Hesselhaus (12) believes that the acid glands alone furnish the toxin, and that the secretion of the alkaline gland serves to neutralize the acid that remains in the sting.

Recent investigations, however, seem to indicate that the venom is secreted only in one gland. This gland is in the average 14 mm. long and has a diameter of 0.19 mm. The gland is forked at its end, and this division into two parts has been the reason for earlier investigators to arrive at the conclusion that two different glands are present. The so-called third venom gland which enters just below the poison-sac seems to be merely a producer of a lubricating substance and does not contribute at all to the venom itself.

Most of the work, although this is not especially mentioned, has obviously been done on the northern European variety of bees, the so-called British Black (*Apis Mellifica Mellifica*), an insect which differs somewhat in color and other characteristics from the so-called Italian race of bees (*Apis Mellifica Ligustica*), which is practically the only variety obtainable in the

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United States. We have obtained our venom entirely from summer bees of the Italian variety. In general, Beck's method was used, and the venom was obtained in a fairly pure form.

We allowed bees to sting into an animal membrane which had been stretched over a small glass vessel containing a sterile solution of 0.9% NaCl solution. Each animal is caught with a pair of pincers and brought in contact with the wet membrane. The animal will sting at once; the drop (about 0.35–0.50 mgms.) of venom liquid dissolves in the liquid, and the sting remains in the membrane. The material is filtered and the small residue extracted once more with hot 0.9% NaCl solution. We used 2000 bees for each experiment. The solutions were then evaporated *in vacuo* to a volume of 20 cc. and slowly poured into 400 cc. acetone. This same method had been employed successfully by K. A. Forster[1] and also by Hahn and Ostermayer (1). The precipitate was centrifuged with acetone and dried *in vacuo*. The yield from 2000 bees was 220 mg. dried acetone precipitate.

The analyses from 6 such lots were as follows:

$$C—43.9–44.5\%$$
$$H—\ 7.4–\ 7.6\%$$
$$N—15.4–14.4\%$$
$$S—\ 1.1\%$$
$$P—\ 0.48\%$$
$$Mg—\ 0.1\%$$

This coincides with the results obtained by Hahn who found that the "crude venom" of their description has about 0.4% Mg. These authors maintain that other metals (Na, K, Fe, etc.) are not even present in traces.

This analysis differs from the one given by Reinert for a "purified bee-venom" through alcohol precipitation mainly in that we have found sulfur in all our acetone precipitates. In the crude liquid venom as it is secreted by the insect without further purification we found considerable amounts of sulfur, in the average 2.7%.

The phosphorous content when obtained by the method described above was 0.48%, but when a different method of extracting the venom from the insects was used, a far larger percentage was obtained when the stings left on the upper side of the membrane were ground, weighed and extracted twice with $1/2 N$ Formic Acid solution, the average phosphorous content was 2.9%. This amount varied very little.

The specific gravity of the crude venom is 1.1313, it is a clear liquid, reacts acid, tastes bitter and has a faint aromatic odor. When dried at room temperature a residue of about 30% is obtained.

Beck was the first to point out that the principal toxic actions of bee-venom might be due to a proteosis. This has been experimentally proven by Hahn and Reinert. Investigations to be published later which we undertook also showed this to be the fact. In conformity with Klopstock and Neter (13), we found that the bee-venom might contain saponins, because small amounts of tannin destroyed the hemolytic action in the same manner as is the case with the saponins. Flury suggested that the saponin-like component of bee-venom might be a link between the albumen-free sapotoxins of animal origin like Crotalotoxin and Ophiotoxin of snake venoms and the toxins of the cantharides group.

SUMMARY.

A physiologically active bee-venom "solid concentrate" obtained by acetone precipitation was analyzed. It contained sulfur.

The minimum lethal dose for intravenous injections into the white mouse was 3.5 Gm. per Kg. bodyweight.

Twenty-two γ solid acetone precipitate hemolize 0.1 cc. rabbit blood erythrocyte suspension. (1–453,000 dilution.)

Twenty γ in physiological salt solution show definite necrotic action when injected intracutaneously.

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[1] Private communication from K. A. Forster.

REFERENCES.

(1) G. Hahn and H. Ostermayer, *Ber. d. Deutsch. Chem. Gesell.*, 69, 2407–2419 (1936).
(2) G. Hahn and H. Leditschke, *Ibid.*, 69, 2764–2766 (1936).
(3) G. Hahn and H. Leditschke, *Ibid.*, 70, 681–684 (1937).
(4) G. Hahn and H. Leditschke, *Ibid.*, 70, 1637–1644 (1937).
(5) M. Reinert, *Festschr. E. C. Barell*, pages 407–421 (1936).
(6) H. Essex, F. Markowitz, and F. C. Mann, *American J. Physiol.*, 94, 209 (1930).
(7) F. Flury, *Arch. exp. Path. Pharmakol.*, 38, 381 (1897).
(8) J. Langer, *Ibid.*, 38, 381 (1897).
(9) Phisalix, *Compt. rend. soc. biol. Paris*, 139, 326 (1904).
(10) B. F. Beck, "Bee-Venom Therapy," D. Appleton-Century, New York (1935).
(11) Carlet, *Compt. rend. acad. Sciences*, 98, 1550 (1884).
(12) F. Hesselhaus, *Zool. Jahrb., Anat. u. Ontog.*, 43, 369–464, 12–22 (1922).
(13) A. Klopstock and Neter, *Biochem. Z.*, 261, 207 (1933).

QUANTITATIVE ESTIMATION OF THE POTENCY OF DIGITALIS BY THE CAT METHOD IN RELATION TO SECULAR VARIATION.*

BY C. I. BLISS[1] AND J. C. HANSON.

Determinations of the acute toxic dose of a drug or poison follow one of two experimental procedures. The first and more common method is to administer different, predetermined dosages to successive groups of animals, so that each individual in a group receives the same dose. The percentage mortality from a given dose is the measure of effect and from a series of such dosages and percentages the dosage-mortality curve and the median lethal dose can be computed. These same values are obtained directly in the second, more specialized procedure, in that the lethal dose is determined separately for every individual. An example is the cat unit for cardiac glucosides, where the drug is infused into the venous system so slowly that the latent period between infusion and cardiac failure is presumably negligible in comparison with the total time of injection. The results, however, are sometimes computed so as to obscure the similarities between the two procedures. Moreover, there are seldom safeguards for secular fluctuations in susceptibility which are known to complicate many comparisons of the LD50 based upon the first method. Such safeguards have been observed for several years in tests of "Digiglusin" (Digitalis Glucosides, Lilly) by the cat method at the Lilly Research Laboratories, and it is of interest to examine the bearing of these results upon the so-called "cat unit" for cardiac glucosides and related drugs.

EXPERIMENTAL PROCEDURE.

The experimental procedure followed the general method first described by Hatcher and Brody (9). Digitalis extract was infused into the femoral vein of the etherized cat at the rate of 1 cc. per minute until the heart stopped. An assay required eight cats, all from the same source, four on the "Digiglusin" Standard and four on a new sample of extract which had been evaluated previously by the one-hour frog method as described in the U. S. Pharmacopœia. In conducting a test, four cats were etherized and prepared on four animal boards for the infusion of one of the two preparations in dilutions of $1:33^1/_3$, the entire process requiring from 10 to 20 minutes. When

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