BIOASSAY OF ACONITE AND ITS PREPARATIONS. 3. THE COMPARATIVE TOXICITY OF TINCTURE AND FLUIDEXTRACT OF ACONITE TO GUINEA-PIGS AND RATS.*

BY JAMES C. MUNCH AND R. I. GRANTHAM.

The U. S. Pharmacopœia (2) method for the bioassay of aconitine and aconite preparations requires that death be produced in at least two of three guinea-pigs weighing between 275 and 325 Gm. following the subcutaneous injection of specified quantities (0.06 mg. of aconitine and 0.4 cc. of tincture per kilogram.). In the preceding paper of this series (1) it was shown that the minimum lethal dose of aconitine subcutaneously injected into white rats was 0.175 mg./Kg. It was suggested that white rats might be used for the preliminary assay of aconitine and presumably of aconite preparations, but no experiments were conducted upon the galenical preparations. It is the purpose of this paper to show the relative toxicities of four tinctures and two fluidextracts of aconite to guinea-pigs and rats by the U. S. Pharmacopœia X subcutaneous method, as well as by intraveritoneal injections.

Subcutaneous injections were made into guinea-pigs and rats in accordance with the technic outlined in U. S. Pharmacopœia X. In making intraperitoneal injections, the animals were placed upon their backs, the skin over the abdomen lifted and the point of a hypodermic syringe inserted at an angle of 45°. Postmortem examination of a number of animals after intraperitoneal injection showed that in no instance had the intestines been perforated.

The final series of experiments were always conducted upon guinea-pigs within the U. S. Pharmacopœia weight range, although in the preliminary tests some of the pigs were outside this range. Similarly, our final conclusions were based upon results obtained with rats weighing between 175 and 225 Gm. although a greater range of weights was used in the preliminary examinations. All the guinea-pigs were obtained from the same breeder; all of the rats were raised in the laboratory from our stock strain.

Tincture "A" was a laboratory sample from a crude drug submitted for purchase. The other tinctures were stored in closed bottles at room temperature for another investigation. They were used as representing older products. Fluidextracts "E" and ' F" were obtained from the same original batch of fluidextract. One-tenth per cent of hydrochloric acid was present in sample "E," whereas "F" contained no added acid.

The entire series of samples was assayed independently and the results obtained in our individual investigations were not disclosed until after all experiments had been completed. The results agreed within the limits of experimental error. The values for the Minimum Lethal Dose (M. L. D) are given in Table I. For comparison the results previously reported on aconitine (1) are included.

Comparing older preparations with freshly prepared ones, all methods of assay show that a larger dose is required to kill. By subcutaneous injection guinea-pigs required 32 times and rats 25 times as large a quantity of tincture "D" as of tincture "A." After intraperitoneal injections the lethal doses to guinea-pigs and rats were increased to 17 and 25 times the original lethal dose. In consider-

* Scientific Section, A. PH. A., Rapid City meeting, 1929.

ing results upon the fluidextracts tested the same increase in minimum lethal doses is noted. Subcutaneously injected, guinea-pigs required 12.5 times as large a quantity and rats 83 times as much of sample "F" as of "E;" intraperitoneally, 8 and 60 times, respectively. While these do not agree very closely, they do show that either subcutaneous or intraperitoneal injections into the rat are in closer agreement with the official method of assay (subcutaneous injection into guinea-pigs) than are results obtained by intraperitoneal injections into guinea-pigs.

TABLE I.

MINIMUM LETHAL DOSE OF ACONITE PREPARATIONS TO GUINEA-PIGS AND RATS.

Preparation.	Age when assayed, months.	Minimum lethal dose—cc./Kg. Subcutaneous. Guinea-pig.	Rat.	Intraperitoneal. Guinea-pig.	Rat.
Tincture A	1	0.30	0.80	0.30	0.60
Tincture B	3	0.35	1.25	0.75	1.25
Tincture C	6	0.60	1.50	0.40	1.50
Tincture D	28	9.5	20.0	5.0	15.0
Fluidextract E	28	0.02	0.015	0.03	0.025
Fluidextract F	28	0.25	1.25	0.25	1.50
Aconitine[1]	..	0.06	0.175		0.10

[1] Milligrams per kilogram.

TABLE II.

RATIO OF MINIMUM LETHAL DOSES OF ACONITE PREPARATIONS: M. L. D. SUBCUTANEOUSLY TO GUINEA-PIGS TAKEN AS 1.0.

Preparation.	Subcutaneous. Rat.	Intraperitoneal. Guinea-pig.	Rat.
Tincture A	2.67	1.0	2.0
Tincture B	3.6	2.1	3.6
Tincture C	2.5	0.67	2.67
Tincture D	2.1	0.5	1.6
Fluidextract E	0.75	1.5	1.25
Fluidextract F	5.0	1.0	6.0
Aconitine	2.9	..	1.67

Table II contains the ratios of the minimum lethal doses by various methods of injection when the values obtained by subcutaneous injections into guinea-pigs are taken as unity. For the rat subcutaneously the ratio is approximately 2.5 for the tinctures, 3 for aconitine. Intraperitoneal injections tend to give a somewhat lower ratio for both guinea-pigs and rats. Results obtained by intraperitoneal injections into guinea-pigs appear quite erratic. Tinctures "C" and "D" showed a ratio of approximately 0.5; the other tinctures and fluidextracts ranged from 1 to 2.

In general the ratio of lethal doses after subcutaneous and after intraperitoneal injections indicates the rate of absorption and of detoxication and elimination. *A priori* it is expected that intraperitoneal injections will be more toxic than subcutaneous. If a product is slowly absorbed following subcutaneous and rapidly after intraperitoneal injection, the ratio of lethal doses is high. As the rate of absorption decreases the lethal doses tend to approach each other. It appears probably that some of the discrepancies obtained in this investigation may be due to selective differences in the absorption of aconitine and its degradation products. This phase of the question is receiving further consideration.

CONCLUSIONS.

1. Approximately 2.5 times as large a quantity of tincture of aconite is required to kill rats as to kill guinea-pigs subcutaneously. A different ratio seems to hold for fluidextract (for a stabilized sample, about 1.0; for a sample not stabilized, about 5).

2. With increasing age, aconite preparations become less toxic to rats and to guinea-pigs. This is interpreted to mean that the hydrolytic cleavage products of aconitine are less toxic.

BIBLIOGRAPHY.

(1) "Bioassay of Aconite and Its Preparations. I. Lethal Dose of Aconitine to Rats," James C. Munch and G. S. Gittinger, JOUR. A. PH. A., 18 (1929), 17–24.

(2) "The Pharmacopœia of the United States of America," Tenth Decennial Revision (1925).

ABSTRACT OF DISCUSSION ON THE FOREGOING PAPERS.

Frank O. Taylor stated there was much misinformation in the literature and if coöperative investigation of drugs is undertaken as advocated in the Chairman's address, aconite deserves early consideration. He also commented on the difference in the toxicity of the tincture and fluidextract.

C. K. Glycart requested information regarding the identification of the true species by the number of stars; he was informed that this was not entirely adequate as a criterion.

H. A. Langenhan inquired relative to the number of aconite species and the extent of their pharmacological study. He was informed that of the 150 species only seven had been studied pharmacologically; different species differ in toxicity, and no pharmacological studies have been filed upon the native American species.

L. W. Rowe commenting on the last paper, stated that tests upon white mice had not shown the divergencies reported upon rats.

E. E. Swanson commented on the effect of alcohol upon the toxicity of aconite solutions.

THE PHYSICAL PROPERTIES OF GUAIACOL.

BY T. S. CARSWELL.

I. INTRODUCTION.

A study of the literature on the physical properties of guaiacol shows wide discrepancies in the data given, particularly in that regarding the melting or crystallizing point. The United States Pharmacopœia X, states that "solid guaiacol melts about 28°" and the Japanese Pharmacopœia (1922), also states that "guaiacol has a melting point of about 28°." The Nederlansche Pharmacopee (1915) gives a melting point of 27.6° to 27.8°. The Pharmacopee Francaise (1927) says that the melting point is 32° and the boiling point 205°. Hager, in "Handbuch der Pharmazeutischen Praxis," Vol. I (1925), 391, states: "Perfectly pure guaiacol forms large, colorless, prismatic crystals with melting point (crystallizing point) 33°, and boiling point 205°." Perkin (*J. Chem. Soc.*, London, 69, 1188) gives the crystallizing point as 28.3°. Behal and Choay (*Bull. de la soc. chim.* (3), 11, 703) give 28.3° and the same value is given by Pushin (*J. Chem. Soc.*, London, 125, 2628–30 (1924)). Denecke (*Z. anorg. allgem. Chem.*, 108, 1–44 (1919)) gives 28.4°. Jaeger (*Z. anorg. allgem. Chem.*, 101, 1–214 (1917)) gives 32°, and the same value is mentioned quite recently by Waser and Sommer (*Helv. Chim.*