The following data are submitted in connection with the taste tests of capsicum

Sample.	Material.	Dilution.	Pungency.
1.	Paprika.....	1–25,000	distinct
	Paprika.....	1–50,000	very slight
8.	Capsicum, East Indies..	1–50,000	plainly perceptible
3.	Capsicum, East Indies[1]..	1–75,000	very decided
15.	Capsicum, Japanese.....	1–50,000	plainly perceptible
16.	Capsicum, Japanese.....	1–50,000	plainly perceptible
11.	Capsicum, West African.	1–50,000	hot
	Capsicum, West African.	1–75,000	decided
12.	Capsicum, Zanzibar.....	1–75,000	decided
13.	Capsicum, Mombassa...	1–75,000	decided
	Piperine, Merck.....	1–50,000	hot
	Piperine, Merck.....	1–100,000	decided
	Capsaicin[2].....	1–1,000,000	very pungent, burning
	Capsaicin[2].....	1–10,000,000	decided

THE ASSAY OF ACONITE.

BY A. R. L. DOHME.*

The work covered by this paper represents what has been done during the past three years by the Scientific Section of the American Drug Manufacturers' Association who have felt that its results should be made known to the medical and pharmaceutical professions generally.

The primary problem was to attempt to decide whether the chemical assay of aconite and its preparations had any real value, and the resultant problem was to determine if the physiological assay was accurate and trustworthy. The present official assay process U. S. P. IX Revision is a chemical one with an alternative physiological assay, but the chemical assay is the standard. In the U. S. P. VIII there was only a chemical assay as the official process. In both cases the end-product was represented by ether-soluble alkaloids. We have shown that ether-soluble alkaloids are not all aconitine but represent a more or less variable proportion of aconitine and its products of hydrolysis benzoyl-aconine and aconine. This variability alone makes the assay process of little value as an absolute standard of therapeutic efficiency and, as well, makes its relative or comparative value more or less of an uncertain quantity.

In order to determine definitely if the three alkaloids which constitute the ether-soluble alkaloids—aconitine, benzoyl-aconine and aconine—could be separated from one another by chemical means a supply of pure aconitine was procured and hydrolyzed into benzoyl-aconine and some of the latter hydrolyzed further into aconine. After thus converting a number of grammes in this way and obtaining

[1] Squibb's powdered, purchased 1918, labelled: "the dried ripe fruit of *capsicum fastigiatum;* the genuine East Indian variety, * * * ;" histologically this presents the characteristic epidermis of African capsicum.

[2] Sample furnished by Dr. Arno Viehoever of the Bureau of Chemistry, U. S. Department of Agriculture, University of Illinois School of Pharmacy.

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a supply of each of benzoyl-aconine and aconine in pure condition, attempts were made to determine if varying solubility in all available solvents or precipitation by all known precipitants might give a method of separating them when contained in a mixture. The result was, however, that no method was discovered by which they could be quantitatively separated, as they showed similar solubilities and precipitation by precipitants. It was, therefore, decided that a chemical separation quantitatively was not feasible, and that the so-called chemical method of assay was not possible, provided our aim was to get as the end-product of our assay only aconitine.

It was also determined by animal experiments that benzoyl-aconine and aconine do not possess the therapeutic properties of aconitine, and that their lethal dose is quite far removed from that of aconitine.

Hence the final conclusion reached was, that the present chemical assay of aconite for ether-soluble alkaloids was misleading and untrustworthy and had better be abandoned.

The next part of our problem was to see if a physiological assay could be developed which would be of some real value in approximately determining the therapeutic efficiency of aconite and its preparations. This, of course, at once opens up the question as to the correctness of a method of assay which has as its criterion and basis the lethal dose or the amount that will kill a definite weight of animal per gramme. Or in simple form, is lethal power a basis for therapeutic efficiency and is one drug that will kill 300 Gm. of guinea pig in a dose of one milligramme twice as efficient therapeutically upon human beings as one that will kill 300 Gm. of guinea pig in a dose of two milligrammes? On this question pharmacologists and physiologists are apt to divide and differ. As lethal dose is the basis used in physiological assay methods, it is probably the only, and hence the best basis available for determining relative therapeutic efficiency of aconite.

Beginning with the pure aconitine crystals we used for making our hydrolysis products for above experiments, our committee sent out samples of same and of a sample of fluidextract of aconite to the physiological chemists of five different laboratories. The prime purpose of this was to determine whether results by such a minimum lethal dose method would be sufficiently close in the hands of varying laboratories and observers to warrant hoping to utilize it in an assay method—assuming, of course, that minimum lethal dose on guinea pigs would be a basis for therapeutic value. The results follow:

	Aconitine cryst.	Fluidextract of aconite root.
Sharp & Dohme	.0000000625 Gm. per Gm. wt. of guinea pig	0.000300
Upjohn	.0000000600 Gm. per Gm. wt. of guinea pig	0.000266
Parke, Davis & Co	.0000000600 Gm. per Gm. wt. of guinea pig	0.000300
Eli Lilly & Co	.0000000625 Gm. per Gm. wt. of guinea pig	0.000275
Norwich Pharmacal Co	.0000000510 Gm. per Gm. wt. of guinea pig	0.000360

If we assume that minimum lethal dose on guinea pigs is a basis for therapeutic efficiency of aconite then these results distinctly indicate that this method is sufficiently trustworthy and efficient to serve as an assay method to determine the therapeutic efficiency of aconite preparations based upon pure aconitine, crystallized, as a standard.

The Scientific Section of the American Drug Manufacturers' Association hence recommend that the chemical assay be dropped for aconite and its preparations and a physiological assay based upon aconitine crystallized, U. S. P., be substituted in its place.

In my laboratory the m. l. d. for benzoyl-aconine and aconine were also determined on guinea pigs in comparison with the aconitine and the following results obtained:

	M. l. d. guinea pig.	
Aconitine cryst.	0.0000000625	Gm. per Gm. wt. of guinea pig
Benzoyl-aconine	0.00002	Gm. per Gm. wt. of guinea pig
Aconine	0.00025	Gm. per Gm. wt. of guinea pig
Fluidextract Aconite Root	0.00003	Gm. per Gm. wt. of guinea pig

These results indicate that aconitine is about 300 times as efficient, *i. e.*, toxic, as benzoyl-aconine and 4000 times as toxic as aconine and at the same time they apparently possess practically none of the characteristic properties therapeutically of the aconitine—as, for instance, producing numbness on the tip of the tongue, etc.

The method employed in these experiments was for the fluidextract of aconite to dilute 1 Cc. thereof to 10 Cc. with 50% alcohol. Use 300 to 400 Gm. guinea pigs and calculate the dose per pig and dilute this with normal salt solution to a total volume of 1.5 Cc. per pig. Inject this into the subcutaneous tissues of the abdomen and take as a lethal dose the smallest amount which will kill within 24 hours.

For the aconitine dissolve 0.1 Gm. in 100 Cc. of 2% acetic acid. Dilute 1 Cc. of this solution to 10 Cc. with distilled water giving a 1:10000 solution of aconitine. Calculate the total dose required for a pig of 300 to 400 Gm. and dilute with normal saline solution to a sum total of 1.5 Cc. and inject as for the fluidextract of aconite. Approximately 0.00000005 per gramme is usually the lethal dose.

With the aconitine, crystallized, above used as a standard it will now remain to establish by comparative tests in various laboratories the extent of agreement reached in the application of above method of physiological assay for fluidextract and tincture of aconite as well as the drug aconite, which latter will of course only be an application of the method of the two fluid preparations, as a liquid extract will have to be prepared to make the assay. This work is now before our Committee on Aconite and will, doubtless, be worked out sometime soon.

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