

these results will add to the confidence of the manufacturers of pituitary solution in the proposed U. S. P. X. standard and method of assay.

BIO-ASSAY OF VERATRUM PREPARATIONS.

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Within recent years it has been concluded by several authorities that the chemical estimation of the alkaloidal content of veratrum preparations is not indicative of corresponding therapeutic value and that a physiological test should be used. As far back as 1905 Houghton and Hamilton (1) published an article on a new aqueous veratrum preparation called "Veratrone" which was "adjusted to one-fourth the strength of the U. S. P. fluidextract by determining the *M. L. D. per Gm. body weight for frogs* of the same species and weight kept under uniform conditions, comparing the results with those obtained from the injection of known quantities of the U. S. P. fluidextract of veratrum viride." Twelve years later, in 1917, Pilcher (2) reported a large number of physiological experiments and concluded that, for practical purposes, the fatal dose for frogs would seem to be satisfactory.

Since 1905, Veratrone has been standardized by the physiological method proposed by Houghton but there are some disadvantages to this method, the chief of which is the indefiniteness of the end-point. Frogs injected with a lethal dose of veratrone may still be just alive 18 or even 24 hours after being dosed. A whole series of frogs given graded doses may be found 15 hours later to be more dead than alive, but still they are not dead and even a skilled technician hesitates about drawing any conclusion. Such a condition necessitates the use of a large number of frogs before a conclusion can be reached and thus renders the assay very expensive in amount of material used and the time required.

The comparative inexpensiveness of the white mouse first caused us to consider it as a suitable substitute for the frog in standardizing veratrum preparations. After using this animal for testing a number of preparations in comparison with the frog several advantages were discovered, the chief one being the rapidity and definiteness with which an assay can be carried out when white mice are used.

The mice are carefully weighed to within 0.5 Gm. and injected intraperitoneally with such a dilution that the total volume of the dose is not greater than 1 cc. The mice are very quickly affected and final results may be read within 30 minutes after injection, as it is very rare that a mouse dies after that period and then it is doubtful whether reliance could be placed in such delayed results.

The table on the following page gives the *M. L. D.* found on frogs and white mice for a number of veratrum preparations:

This series of tests has shown several facts when the results are carefully analyzed.

The *M. L. D.* of an average or standard *F. E. Veratrum Viride* should be 0.00025 cc. per Gm. body weight of white mouse administered intraperitoneally. Veratrone, an aqueous solution of veratrum adjusted to one-fourth fluidextract strength should have an *M. L. D.* for white mice of 0.0010 cc. per Gm.

Sample.	M. L. D. frogs.	No. of frogs used.	M. L. D. to white mice.	No. of mice used.
Veratrone Rx 728,660	0.0030 cc. per Gm.	30	0.0010 cc. per mg.	20
Veratrone Rx 069,133	0.0025 cc. per Gm.	20	0.0015 cc. per mg.	18
Veratrone Rx 071,523	0.0025 cc. per Gm.	25	0.0010 cc. per mg.	10
Veratrone Rx 072,584	0.0030 cc. per Gm.	35	0.0008 cc. per mg.	7
Veratrone Rx 752,757	0.0018 cc. per Gm.	35	0.0009 cc. per mg.	19
Veratrone Exper.	0.0035 cc. per Gm.	25	0.0020 cc. per mg.	17
Veratrone Exper.	0.0025 cc. per Gm.	25	0.0010 cc. per mg.	15
F. E. Ver. Vir. Rx. 746,868	0.0008 cc. per Gm.	20	0.00025 cc. per mg.	16
Veratrone Dil. Sample A.	0.012 cc. per mg.	24
Veratrone Dil. Sample B.	0.0055 cc. per mg.	11

The number of mice required for an assay is definitely less than the number of frogs required for an assay the average being about three-fourths.

The period of time required for an assay when mice are used is much shorter than that necessary when frogs are used.

The mouse method is less expensive and more practical than the frog method.

In order to determine definitely whether the mouse test is fully as accurate as the frog test, when each is properly applied, a series of two unknowns were tested on white mice. These two samples represented definite dilutions made from two of the samples tested above and were entirely unknown to the technician who conducted the test until after the result was definitely determined. Sample "A" was a 1 to 8 dilution of Rx 069,133. Since Rx 069,133 had an M. L. D. of 0.0015 cc. per Gm. to mice a 1 to 8 dilution should have an M. L. D. of 0.012 cc. per Gm. and this is the result reached experimentally. Sample "B" was a 1 to 5 dilution of Rx 071,523. This Rx had an M. L. D. of 0.0010 cc. per Gm. so a 1 to 5 dilution should have an M. L. D. of 0.0050 cc. per Gm. The result determined experimentally was 0.0055 cc. per Gm. which was but 10% higher than it should be.

The test of these unknowns has demonstrated the accuracy of the method.

With these several points in its favor the Bio-Assay of veratrum preparations by determining their M. L. D.'s to white mice is a natural forward step.

BIBLIOGRAPHY OF VERATRUM BIO-ASSAY LITERATURE.

- (1) Houghton & Hamilton, *Ther. Gazette*, Jan., p. 1, 1905.
- (2) Pilcher, *Amer. Jour. Phys.*, 44, 1, 1917.

FROM THE MEDICAL RESEARCH LABORATORIES,
PARKE, DAVIS & CO., DETROIT.

THE U. S. AND BRITISH OPIUM PROPOSALS.

America wants opium smoking stopped entirely within ten years. Great Britain, through Lord Cecil of Chelwood, announced that she stood ready to stop it in fifteen years, but conditionally,

Britain's condition is that the fifteen years shall begin to run only from the time when China will have reduced the raising of opium to such a stage as "will remove the danger of opium smuggling from China into other Far Eastern territories."

The American position is that the danger of smuggling may continue to exist indefinitely, thus postponing forever the inauguration of the British suggestion.