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THE BIOASSAY OF VERATRUM VIRIDE.*,[1]

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Although the U. S. P. X recognizes no chemical or biological assay for *Veratrum viride*, it is a well-known fact that the preparations of this drug vary greatly in potency (1, 2). This variation in potency and the lack of a satisfactory assay method seem to be the only explanation for the fact that this drug has been almost completely discarded.

A chemical assay has been suggested (3, 4), but since the activity of *Veratrum viride* is due to a number of alkaloids which differ qualitatively and quantitatively in action (5) it would appear that a chemical assay would not be sufficient. This has been pointed out by several workers (1, 6).

Houghton and Hamilton (7) reported a biological assay of *Veratrum viride* based upon the M. L. D. per Gm. body weight of frog. Pilcher (2) did further work with this method and concluded that for all practical purposes the frog assay appeared to be satisfactory.

Rowe (8), in reporting a biological assay based upon the M. L. D. for white mice, made the following comment on the indefinite end-point of the frog method and the length of time required: "A whole series of frogs given graded doses may be found fifteen hours later to be more dead than alive, but still they are not dead and even a skilled technician hesitates about drawing any conclusions." In our experience the mouse method has the same objection. The advantages claimed for the mouse method were that fewer animals were needed and that the assay required less time. Pilcher (2) used an M. L. D. for guinea pigs but preferred the frog method.

Veratrum viride is a centrally acting cardiac depressant. In therapeutic doses it has a selective stimulating action on the vagus nerve (9). It has long been known that large doses cause emesis. Some workers claim that the emesis is central, while some claim that it is local. Hanzlik (10) states that the emesis of Veratrum is the result of local irritation, and that it is produced by intraperitoneal but not by intravenous injections.

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Since it appears that a chemical assay for *Veratrum viride* is not sufficient, and since the biological assays presented require long periods of observation and lack definite and easily observed end-points, it is evident that a more satisfactory assay method is needed. In view of the fact that Veratrum is a medullary stimulant, that the vomiting centers are located in the medulla and that emesis usually results from the administration of large doses, it is quite probable that this emesis is a result of the central medullary stimulation, that it is a manifestation of the desired pharmacological action and that it would furnish a satisfactory basis for a biological assay. We have found that small and very definite doses of Veratrum consistently produced emesis upon intravenous injection in pigeons. We also found, in our first experiments, that we could determine the emetic dose of a tincture within a range of 0.005 cc. per Kg. body weight of pigeon. This accuracy, the fact that emesis results within only a few minutes after administration, that the technique was very simple, that the animals were not killed and could be repeatedly used, suggested this as an ideal assay method.

In this preliminary work the potencies of six tinctures of *Veratrum viride*, U. S. P. X, were compared, using the average minimum emetic dose for pigeons as the basis for comparison. Three of these, No. 102, No. 104 and No. 105, were commercial tinctures prepared by reliable manufacturers. Two of these, No. 102 and No. 104, were claimed by the manufacturer to assay 0.1 per cent total alkaloids. The other tinctures, No. 110, No. 111 and No. 112, were prepared by the U. S. P. X process from the crude drug obtained from reputable drug houses.

In order to compare the effect of the method of administration upon the emetic dose, the M. Em. D. of one tincture, No. 112, was also determined by intraperitoneal injection. The intravenous method seemed much superior and was, therefore, used with the six preparations included in this work.

The minimum emetic dose was considered as the smallest dose, expressed in cc. of the tincture per Kg. body weight, which would produce emesis within fifteen minutes in approximately 75 per cent of the pigeons injected.

The method used was very similar to the one proposed by Hanzlik (10) for estimating the potency of digitalis. Adult pigeons weighing, roughly, from three to four hundred grams were used. Freshly prepared dilutions (1 to 20 and 1 to 40) of the tincture in physiological salt solution were injected into the wing veins, and the pigeons placed in individual cages and observed for a period of fifteen minutes. A longer period of observation was found to be unnecessary. The emesis which resulted, usually in from two to five minutes, was of short duration and was characterized by a downward craning movement of the head and a convulsive flapping movement of the wings. There was usually an expulsion of gravel or mucus.

In determining the emetic dose for each preparation two doses of a wide range were first given to "bracket" the emetic dose, and then by a series of injections of doses within this range the minimum emetic dose was approached. Since the doses well below the emetic dose failed to produce emesis in almost every case and the doses above the emetic dose in all but one case, No. 110, caused emesis, only the emetic doses and the doses just below the emetic dose have been included in the tables.

The results obtained in determining the average minimum emetic dose of six tinctures of *Veratrum viride* intravenously, the results of one determination using

intraperitoneal injection, and a comparison of the potencies of the six tinctures based upon this method, are presented in the following tables:

TABLE I.—THE AVERAGE MINIMUM EMETIC DOSE OF SIX TINCTURES OF VERATRUM VIRIDE.

Preparation Number.	Dose Cc./Kg.	Number of Injections.	Emesis.	No Emesis.
102	0.060	7	1	6
	0.065*	11	10	1
104	0.050	5	0	5
	0.060*	11	8	3
105	0.020	10	3	7
	0.030*	7	7	0
110	0.020	14	7	7
	0.025*	5	5	0
	0.030	8	6	2
111	0.020	10	4	6
	0.030*	7	6	1
112	0.020	11	4	7
	0.030*	6	6	0

* Minimum Emetic Dose.

TABLE II.—A COMPARISON OF THE AVERAGE MINIMUM EMETIC DOSE OF THE SIX PREPARATIONS TESTED. ALSO, A COMPARISON OF THE POTENCIES OF THE PREPARATIONS AS INDICATED BY THE DIFFERENCE IN THE SIZE OF THE INTRAVENOUS EMETIC DOSE FOR PIGEONS.

(For Convenience of Comparison, Preparation 102 Is Indicated as Having a Potency of 100 Per Cent.)

Preparation.	M. Emetic Dose Cc./Kg.	Comparative Potency.
102	0.065	100%
104	0.060	108%
105	0.030	217%
110	0.025	260%
111	0.030	217%
112	0.030	217%

TABLE III.—THE INTRAPERITONEAL EMETIC DOSE OF PREPARATION NO. 112.

(This Is the Only Case in Which Intravenous Injection Was Not Used.)

Dose Cc./Kg.	Number of Injections.	Emesis.	No Emesis.
0.040	4	0	4
0.060*	4	3	1

* Minimum Emetic Dose.

DISCUSSION AND CONCLUSIONS.

As shown by Table II, there was a wide variation in the potencies of the tinctures tested as indicated by the difference in the size of the intravenous dose necessary to produce emesis in pigeons. Preparations No. 102 and No. 110 (Tables I and II), prepared by the U. S. P. X process, showed a variation in potency of more than 100 per cent.

Although the time required for emesis to take place was not given in the preceding tables, the average time required for emesis following intravenous injections was about five minutes. In no case did emesis occur after fifteen minutes.

For the purpose of comparing methods of administration, the minimum emetic

dose of preparation No. 112 was determined by intraperitoneal injection (Table III). It was found that approximately twice the intravenous dose (Table I) was required to produce emesis when the drug was administered by this method. It was also found that approximately twenty minutes was required for emesis to take place following intraperitoneal injections.

No attempt was made to determine the seat of the emetic action; however, the following data indicate that emesis is the result of a central action rather than the result of local irritation as claimed by Hanzlik (10): 1. Intravenous injections of the drug produce emesis usually in from two to five minutes. 2. About twenty minutes is required for an emetic action to take place when the drug is administered intraperitoneally. 3. The intravenous emetic dose is much smaller than that dose required to produce emesis when injected intraperitoneally. 4. The consistency obtained would hardly have been possible had the emetic action been due to local irritation. The fact that *Veratrum viride* is known to act upon the medullary center and that the vomiting centers are located in this part of the brain further substantiate these indications.

From the consistency obtained in this preliminary work it appears that the minimum emetic dose for pigeons might furnish a satisfactory basis for a biological assay of this drug; however, further work is being done in an attempt to definitely determine its reliability.

If it can be shown that the pigeon emesis method gives a reliable measure of the desired pharmacological activity of *Veratrum viride*, it would have the following attributes of a good biological assay method: 1. A very definite and easily recognized end-point. 2. Economy. The pigeons may be repeatedly used. 3. Little time is required. The maximum period of observation is fifteen minutes. With the frog method, which seems to be in greatest favor, this period may exceed twenty-four hours. 4. An accuracy of within 10 per cent. 5. Simplicity. The technique is very simple and does not require the aid of an assistant.

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STORY OF LIFE AT TEXAS CENTENNIAL.

"The Story of Life," a coöperative exhibit created at a cost of $100,000.00, which will occupy more than 10,000 square feet in the great hall of the Federal Government, promises to be one of the outstanding attractions of the Texas Centennial Exposition.

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