BIOLOGICAL ASSAY OF GELSEMIUM.*,[1]

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INTRODUCTION.

Although the rhizomes and roots of Gelsemium sempervirens have been used as a medicinal agent for a great many years there has not yet been presented an acceptable means for the standardization of its preparations.

A number of workers, including Kollock (1), Gerrard (2), Thompson (3), Sayre (4), (5), (6), (7), and recently Chen, Pak and How (8), have investigated the constituents of this drug and have reported different alkaloids. However, there still exists confusion as to the number and names of these alkaloids. Also, there still exists uncertainty as to the action of these different alkaloids.

Manufacturers use total alkaloidal content as an indication of the strength of the drug but the fact that the Revision Committee of the N. F. VI failed to accept an assay based upon total alkaloids denotes that this is not believed to be a true indication of the physiological activity of the drug.

Githens and Vanderkleed (9) suggested as a means of assay the determination of the minimum lethal dose for guinea pigs, showing that there was a wide variance between the M. L. D. and total alkaloids. This conclusion was further indicated by the work of Pittenger (10) who stated that the determination of M. L. D. for guinea pigs was a suitable means of assay. Swanson and Hargreaves (11) suggested the determination of M. L. D. on white mice as a means of standardization also showing that the total alkaloidal content did not run parallel with this M. L. D.

Since neither the chemical nor the physiological methods for assay so far proposed have proved satisfactory, the question presented itself as to whether other means of physiological action might not be used for standardization. It was found through preliminary investigations that intravenous doses of Tincture of Gelsemium consistently produced emesis in pigeons. Assuming that this emesis is a demonstration of the pharmacologic activity of the drug, this work was undertaken to determine whether this emetic action could be utilized as a means of standardization. After determining whether the method was feasible or not, several preparations were studied to determine if any parallelism existed between the emetic dose and the total alkaloidal content of the tincture.

No attempt was made in this study to determine the cause of the emetic action, but it seems logical to assume that this is central. This assumption is based upon the fact that it has been pointed out by Chillingsworth (12) that Gelsemium slows the heart action by way of the vagus. Since the seat of the emetic action is thought to be in the medulla, and since the action of this drug is prominent in that region, the conclusions seem justified. The fact that emesis is produced rapidly and consistently when intravenously injected further lends weight to this opinion. Since the therapeutic use of the drug is based upon its central action, if the emetic action is central this would seem to be justification enough for employing such action as a means of standardization.

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EXPERIMENTAL PROCEDURE.

Six preparations were used in this study, these being designated by the numbers 101, 102, 104, 106, 107 and 108. These are tinctures with the sources as indicated in Table I.

TABLE I.—TABULATION OF DATA CONCERNING GELSEMIUM PREPARATIONS USED IN EXPERIMENTAL TESTS.

Preparation Number.	Menstruum.	Assay Gm. Total Alkaloids per 100 Cc.	Source.
101	N. F. V	0.045–0.055	Manufacturer
102	Approx. 60 % alcohol		Prepared from fresh drug, cultivated in Florida (100 cc. represents 10 Gm. of dried drug)
104	N. F. VI		Prepared from a mixture of 5 samples crude drug obtained on the market
106	N. F. VI		Same drug as 102 after careful drying
107	N. F. V	0.045–0.055	Manufacturer
108	N. F. V	0.055	Manufacturer

Method for Physiological Assay.—The procedure followed is similar to that suggested by Hanzlik (13) for the standardization of Digitalis.

The pigeons used were mature birds ranging in weight from two to four hundred grams. They were starved for approximately twelve hours prior to injection. Immediately before injection the pigeon was weighed to the nearest five grams and the dose calculated in cc. per Kg. of total weight.

Solutions for injection were prepared by diluting with physiological saline solution; equal parts saline solution and tincture in some cases and others two parts saline solution to one part tincture. To preclude the possibility of any material settling out after dilution, only such amount as would be used in making five or six injections was diluted at a time.

For injection the feet of the pigeon were secured to a table with a heavy twine. The wing to be injected was held in the left hand in such a manner that it could be spread out and at the same time have a finger resting against the body. The injection was then made and the bird placed in an individual cage for observation.

Alkaloidal Determination.—For the determination of the amount of total alkaloids contained in the tinctures a procedure similar to that used by Pittenger (10) was employed. At a temperature of 60° C., 100 cc. of the tincture was concentrated to a volume of about 15 cc. This was diluted with water to about 80 cc., and the volume made up to 100 cc. with solution of Lead Subacetate. This was filtered as clear as possible and the excess lead was precipitated with Exsiccated Sodium Phosphate. This was filtered, 50 cc. placed in a separatory funnel, and made alkaline with Solution of Amonium Hydroxide. The alkaline solution was then shaken with successive portions of chloroform, using 20, 20, 15 and 10 cc. The combined portions of chloroform were then dried and weighed.

Each sample was determined in duplicate. Due to the fact that there was not sufficient quantity of Preparations 101 or 102 available no alkaloidal determination was made for these. For Preparations 107 and 108 the samples taken were but 50 cc. The procedure followed was the same and calculations made accordingly. The results were expressed in grams of crude alkaloids per 100 cc.

Experimental Data.—A comparison of the strengths of six tinctures was made by a determination of their minimum emetic doses. The Minimum Emetic Dose (M. Em. D.) is that amount of tincture, expressed in cc. per Kg. of total body weight, which will produce emesis in 75 per cent of the pigeons intravenously injected, within 30 minutes.

Although a large number of injections were made it is not possible to show all the results here, but Table II is an expression of the M. Em. D. of the six tinctures with the dose just above and that just below shown in each case. The time for emesis is not shown in this table, but 30 minutes was chosen as a maximum, the emesis usually occurring in from 3 to 16 minutes. The remaining tables, III, IV and V are self explanatory.

TABLE II.—MINIMUM EMETIC DOSE OF EACH OF THE SIX TINCTURES WITH THE RESULTS OF INJECTIONS OF THE DOSE IMMEDIATELY ABOVE AND BELOW.

Preparation.	Dose, Cc./Kg.	Number of Injections.	Results of Injections. Emesis.	No Emesis.
101	0.95	20	16	4
	0.90*	20	15	5
	0.85	20	13	7
102	0.55	8	7	1
	0.50*	16	12	4
	0.45	8	4	4
104	0.95	8	8	
	0.90*	16	13	3
	0.85	12	7	5
106	0.35	12	10	2
	0.30*	12	11	1
	0.25	8	4	4
107	0.75	8	6	2
	0.70*	12	9	3
	0.65	8	4	4
108	0.80	8	6	2
	0.75*	8	8	
	0.70	8	4	4

* Minimum Emetic Dose.

TABLE III.—A COMPARISON OF THE M. EM. D. OF THE SIX TINCTURES. THE STANDARD PREPARATION, 104, IS TAKEN AS 100%.

Preparation.	M. Em. D.	Comparative Potency.
101	0.90	100%
102	0.50	180%
104	0.90	100%
106	0.30	300%
107	0.70	128%
108	0.75	120%

TABLE IV.—ALKALOIDAL ASSAYS: GRAMS OF TOTAL ALKALOIDS PER 100 CC. OF TINCTURE.

Preparation.	Sample 1.	Sample 2.	Average.
104	0.0473	0.0466	0.047
106	0.1190	0.1143	0.117
107	0.0638	0.0640	0.064
108	0.0434	0.0458	0.045

TABLE V.—A COMPARISON OF TOTAL ALKALOIDS IN GRAMS PER 100 CC. AND THE M. EM. D. OF FOUR OF THE SIX TINCTURES.

Preparation.	Total Alkaloids.	M. Em. D.
104	0.047	0.90
106	0.117	0.30
107	0.064	0.70
108	0.045	0.75

DISCUSSION.

The injections of a certain dose were made in groups of four pigeons and although the results of a group of four would occasionally vary from that finally obtained, it was found that the results from eight injections could always be taken as the expression of that dose. The M. Em. D. could then be verified by injecting another group of four.

In considering the question as to whether pigeons could be used over and over again a large number of injections were made on a small group of birds allowing them a period of about two weeks between injections. Then the same dose of the same tincture was injected into a number of pigeons that had received no previous injections with results that did not materially differ from that obtained with the group that had been injected a number of times. To further debar the idea that emesis may result from a sensitiveness to injection a number of controls were run. Injections were made with saline solution having the same alcoholic strength as the dilutions of the preparations being used. The dose of the control injection was the same as the largest dose of the preparation being used. The pigeons used for controls were from a group which had received the largest number of injections. In no case did such an injection produce emesis.

It is not believed that weight of pigeon, within the range used, is a factor to be considered, because in checking the large number of injections made in this study no noticeable difference could be detected as due to weight.

There is a definite variation in potency in the six preparations tested (see Table III). With reference to the three preparations made by the method of the N. F. V (101, 107, 108), it can be seen that there is a difference of 28 per cent between the largest and the smallest M. Em. D. Since Preparations 102, 104 and 106 were made by methods other than that of the N. F. V it is not possible to compare these directly with the other tinctures, but it can be seen that there is a wide variation in the M. Em. D.

Two preparations were made from the same crude drug (Table I), one (102) while the drug was fresh, the other (106) after it had been carefully dried. There is a difference in their M. Em. D., the preparation from the dried drug being the stronger. Sayre (14) has pointed out that if it is carefully dried, there will be little difference in the total alkaloidal content of the fresh and the dried drug. Since both these tinctures represent the same quantity of the dried drug, the difference in strength is probably explained by the fact that the drug of Preparation 102 was not reduced to as fine state as that of 106, and by the fact that the alcoholic strength of the menstruum of 106 was greater than that of 102. The results from these two tinctures indicate that the cultivated plant in Florida produces a much stronger drug than that ordinarily found on the market.

Referring to Table V, it can be seen that Preparations 104, 106 and 107 show a rather close parallelism between the total alkaloids and the M. Em. D. But Preparation 107 does not show the same parallelism. The number of preparations here is too small for any definite conclusions but it does tend to indicate that the total alkaloidal content and the M. Em. D. do not always run parallel.

CONCLUSIONS.

The need for a more acceptable assay for Gelsemium has again been pointed out and this is further indicated by the differences found in the strengths of the preparations tested. The indication that alkaloidal content and physiological activity are not always parallel further points to this need.

It is indicated from the data obtained in this study that the determination of the minimum emetic dose in pigeons might serve as an adequate measure of physiological activity; however, further study will be necessary to substantiate this belief.

There is an indication that the drug gathered from a cultivated plant in Florida is much more potent than that usually found on the market. This is suggested as a point for further study.

Should the determination of the M. Em. D. prove a satisfactory means of measuring pharmacological activity, the method would possess the following desirable qualities: economy, simplicity (an assistant being unnecessary), rapidity and a definite end-point.

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NEW YORK BOTANICAL GARDEN.

A report of the New York Botanical Garden speaks of extensive additions. The beauty of the Garden will be enhanced by more than a thousand trees and shrubs and 800 young hemlocks; 7000 heathers will be added to the heath planting.

SMITHSONIAN INSTITUTION.

The report of Secretary Charles G. Abbott of the Smithsonian Institution states that "nearly half a million specimens were added to the collections of the National Museum, mostly as gifts or from Smithsonian expeditions." Besides the usual scientific publications, a weekly radio broadcast on the activities of the institution has been put on the air by the Office of Education in coöperation with the National Broadcasting Company.—Through *Science*.