themselves: First, that of seasonal variation. The experiments with the larger doses were performed during the summer, at which time, in the case of the other drugs, such as the digitalis group, absorption is most difficult.[1] Second, another possible reason for it is the well-known styptic effect of epinephrin on the blood vessels locally. The stronger solutions might, in this way, retard their own rate of absorption, and thus not show the characteristic pupillary effect more promptly than a weaker solution which would not impede its own rate of absorption to as great a degree. Third, another factor operative in such a contingency, would be the well-known tendency for epinephrin to undergo rapid loss of activity, so that if its absorption should be interfered with in the manner suggested, that which enters the circulation would tend to be disposed of faster than additional amounts could enter, and thus the latent period would, of necessity, be lengthened until such time as absorption more than offsets elimination.

It is not intended in this connection to lay any particular emphasis upon the value of these experiments with reference to any action of epinephrin itself, but only in so far as they confirm our findings mentioned above with regard to the digitalis drugs.[1] If it is borne in mind that the official method of assay for the digitalis group requires that the drug be injected into the lymph sac, and that, as is well known, many samples cannot be successfully assayed by the method, on account of the fact that they are poorly absorbed from the lymph sac, the significance of our experiments becomes more apparent, as a further proof of the unreliable character of the present official method. However it may be stated that no such variability in the rate of absorption from the lymph sac was found in the case of epinephrin as in the case of digitalis.

In our previous article[1] on this question, we submitted much evidence to show that if the same size doses of digitalis were, instead, injected into the muscles a much more constant and positive result followed. Because of the very great pharmaceutical and therapeutic importance of the question involved, it seemed desirable to obtain still further evidence of the superiority of the intramuscular method in comparison with the official method. The results of the present experiments are offered, therefore, as further support of this fact developed in our earlier experiments.

REFERENCES.

[1] M. S. Dooley and C. D. Higley, JOUR. A. PH. A., 11, 911, 1922.
[2] Meltzer and Auer, *Am. Journ. Physiol.*, 11, 449, 1904.

THE STANDARDIZATION OF GELSEMIUM.*

BY PAUL S. PITTENGER.

Although the amount of Veratrum and Gelsemium prescribed and used by the present-day practitioner is very small as compared with Aconite and some other cardiac depressants, these drugs are still used in appreciable quantities.

As stated in a recent paper,† "It is the opinion of the author that any drug

* Scientific Section, A. Ph. A., Asheville meeting, 1923.

† Pittenger, "The Standardization of Veratrum," read before the Scientific Section of the Penna. Pharm. Association, Bedford Springs, Pa., June 1923.

which is worthy of being used as medicinal agent should be standardized, if possible, by either chemical or biologic methods.

"Since the principle end to be accomplished by the assay of the drug or its preparations is to secure a *means of measuring* its therapeutic efficiency, a chemical method fails of its purpose unless some direct and constant ratio exists between the results obtained by the assay process and the therapeutic activity of the drug.

"For this reason a chemical assay is of no value unless the results obtained by the chemical assay parallel the results obtained by the biologic assay.

"Without a satisfactory biologic method it is almost impossible to determine whether or not the substance isolated by chemical methods bears any relation to the activity of the drug.

"For this reason I was interested in making a comparison between the results of the chemical and physiological assays of this drug and its preparations."

Chemical Investigation.—The chemistry of Gelsemium has been made the subject of a large number of investigations including the work of Kollock,[1] Maisch,[2] Eberle,[3] Wormley,[4] Coblentz,[5] Gerrard,[6] Thompson,[7] and others. These researches show that the drug contains two alkaloids, Gelsemine and Gelseminine.

In view of the fact that two different alkaloids are present, it was decided to determine whether or not an estimation of the total alkaloid would be an index to the therapeutic activity of Gelsemiun and its preparations.

With this object in view the following chemical methods were employed:

GELSEMIUM DRUG.

Assay for Alkaloids.—20 Gm. percolate with alcohol to exhaustion, concentrate to about 20 cc, dilute to 80 cc with water, add lead subacetate solution *q. s.* 100 cc. Filter off all possible, remove excess of lead with 2 Gm. dry Na_2HPO_4. Filter off 50 cc, make alkaline with NH_4OH, shake out with $CHCl_3$, evaporate, dry and weigh.

TINCTURE AND FLUIDEXTRACT GELSEMIUM, U. S. P.

Assay for Alkaloids.—Samples fluidextract 20 cc; samples tincture 200 cc. Finish like drug.

SOLID EXTRACT AND POWDERED EXTRACT GELSEMIUM.

Dissolve 4 Gm. in alcohol and finish like drug.

Tentative Chemical Standards.—By applying these chemical methods to the above samples of Gelsemium and its preparations, results were obtained leading to the adoption of the following tentative, chemical standards:

Drug	0.4 % total alkaloids
Tincture	0.04% total alkaloids
Fluidextract	0.4 % total alkaloids
Powdered Extract	2.0 % total alkaloids

Physiologic Action.—According to Cushny, Gelsemine is only slightly active, induces the same symptoms in frogs as Strychnine, but having no effect on mammals

[1] Kollock, *A. J. P.*, XXVII.
[2] Maisch, *A. J. P.*, 1869.
[3] Eberle, *A. J. P.*, 1869.
[4] Wormley, *A. J. P.*, 1870.
[5] Coblentz, PROC. A. PH. A., 1897, p. 225.
[6] Gerrard, *A. J. P.*, 1883, p. 258.
[7] Thompson, *Pharm. Era*, 1887, p. 3.

even when injected into a vein in very large quantities. Gelseminine, on the other hand, is a powerful poison which resembles Coniine in most of its effects.

The action of Gelsemium is, therefore, undoubtedly due to Gelseminine and not to Gelsemine, as far as mammals are concerned.

In view of the fact that the drug possesses a depressant action, the method employed for assaying Aconite and Veratrum on guinea pigs presents itself as a likely means of physiologic standardization. Therefore, about 14 years ago I carried out a series of experiments by making definite dilutions of fluidextract Gelsemium and then determining the smallest amount of the preparation per 250 Gm. body weight of animal required to cause the death of the animal within 24 hours when the preparation was subcutaneously injected.

It was found that the M. L. D. as determined by this method was in direct proportion to the dilution.

The experiment was repeated several times and it was found in each instance the M. L. D. paralleled the actual dilution.

As a result of these experiments we adopted the following method for the biologic standardization of Gelsemium:

BIOLOGIC ASSAY METHOD.

Animals.—Guinea-pigs in good physical condition and weighing from 180 to 400 Gm.

Preparation of Experiment.—The guinea-pigs are prepared for the injection by clipping or shaving the hair from about one square inch of the skin over the abdomen and painting the exposed portion with 5% Tr. Iodine. The pigs are then weighed and records kept.

Method of Injecting.—Injections are given subcutaneously in the abdominal region. In all cases the preparation should be sufficiently diluted, or concentrated, as the case may be, to make the dose injected measure not less than 0.5 cc nor more than 4 cc.

Actual Standardization.—Into a series of 4 guinea-pigs, inject 9/10, 10/10, 11/10, and 12/10 of the standard dose of the preparation to be standardized for each 250 Gm. body weight of guinea-pigs. The animals are then placed in cages and allowed to remain for twenty-four hours, when they are examined and a note made of those living and those which are dead.

The results of this preliminary test, in which the range of dosage is quite wide, enable the investigator to form some idea as to the strength of the preparation. Basing the dosage upon these results, other series of guinea-pigs are injected with progressively increasing or decreasing doses, as the case may be, still further diminishing the variation between doses, until the smallest amount is found which will prove fatal within twenty-four hours. The probable minimum lethal (toxic) dose of the preparation, unless it deviates considerably from that of the standard, is generally obtained by one or two series of injections. In order to determine whether or not this is the true minimum lethal dose, this result is checked by carefully injecting a new series of four pigs; two with the smallest dose that was found to kill, and two with the largest dose that did not kill. If, however, any of this last series show irregularities, further correction must be made.

Tentative Biologic Standards.—In order to determine the average M. L. D. of the drug and its various preparations, and thus set a tentative standard for assay

purposes, assays were made of all available samples of drug, and samples of the galenical preparations purchased from the different pharmaceutical manufacturing houses in the U. S.

As a result of these assays, the following tentative standards were adopted:

M. L. D. PER 250 GM. BODY WEIGHT OF GUINEA PIG.

Drug (in the form of Fluidextract).....	0.375
Tincture.....	2.5
Fluidextract.....	0.375
Powdered Extract.....	0.125

The assays showed a wide variation in the M. L. D. and, therefore, in the strengths of the various commercial preparations on the market and proved the necessity for standardizing preparations of this drug.

COMPARISON OF RESULTS OBTAINED BY THE CHEMICAL AND BIOLOGICAL METHODS.

In order to determine whether or not the results obtained by the above chemical methods would parallel results of the biologic assay it was decided to test different samples by both methods. Therefore, during the past 14 years, samples have been taken at different intervals and tested.

The results of these tests follow:

TINCTURE GELSEMIUM.

Sample.	Chem. Assay. Per cent.	Bio. Assay. Per cent.	Date.	Sample.	Chem. Assay. Per cent.	Bio. Assay. Per cent.	Date.
1	106	100	6-21-09	25	40	125	2- 9-14
2	98	75	9-13-09	26	35	55	2-27-14
3	130	200	10-18-09	27	97	200	3-16-14
4	175	200	10-18-09	28	85	208	5- 4-14
5	180	166	11-30-09	29	95	138	9- 3-14
6	134	125	2-10-10	30	57	200	12-11-14
7	122	111	7-11-10	31	102	125	5-10-15
8	139	111	9-12-10	32	121	138	11-18-15
9	78	97	11-24-10	33	45	138	11-29-15
10	100	83	12- 2-10	34	125	111	6- 6-16
11	140	178	12-16-10	35	135	131	12-26-16
12	115	156	1- 3-11	36	205	312	12-26-16
13	102	69	2-15-11	37	125	90	2- 9-17
14	142	66	4-12-11	38	97	125	7- 5-17
15	96	83	7- 1-11	39	110	108	3-11-18
16	120	100	10-15-11	40	135	250	2- 8-19
17	102	111	1-17-12	41	110	138	2-22-19
18	171	90	2-14-12	42	130	138	8- 8-19
19	125	125	5-27-12	43	75	125	1-30-20
20	128	125	1-11-13	44	157	125	5-22-20
21	107	192	1-28-13	45	170	166	7- 1-20
22	155	200	6-20-13	46	150	208	7- 1-20
23	127	142	7-22-13	47	180	166	7- 1-20
24	134	208	7-22-13	48	90	138	6-15-21

FLUIDEXTRACT GELSEMIUM.

Sample.	Chem. Assay. Per cent.	Bio. Assay. Per cent.	Date.	Sample.	Chem. Assay. Per cent.	Bio. Assay. Per cent.	Date.
1	115	150	4-22-09	5	100	200	11-30-09
2	100	150	5-25-09	6	100	100	12-13-09
3	125	65	7- 6-09	7	137	117	2- 4-10
4	112	250	10-18-09	8	116	94	2- 5-10

Sample.	Chem. Assay. Per cent.	Bio. Assay. Per cent.	Date.	Sample.	Chem. Assay. Per cent.	Bio. Assay. Per cent.	Date.
9	103	75	4–27–10	26	105	115	6–12–13
10	134	120	8–11–10	27	151	151	7– 7–13
11	110	100	8–18–10	28	109	125	9–17–13
12	110	125	10–31–10	29	95	151	12– 4–13
13	74	68	2–15–11	30	73	54	5–23–14
14	112	94	2–16–11	31	113	187	5–10–15
15	129	140	5–18–11	32	62	187	12–14–15
16	171	94	7– 1–11	33	130	151	7– 7–16
17	187	215	8–24–11	34	110	215	2– 1–17
18	99	166	11–28–11	35	120	215	3– 6–17
19	104	94	3– 7–12	36	235	312	12–21–17
20	141	140	6– 3–12	37	150	151	12– 5–18
21	145	187	8– 3–18	38	107	187	4– 1–19
22	96	115	1–11–13	39	80	94	10–21–20
23	8	54	2–17–13	40	100	125	11– 8–20
24	80	54	5–10–13	41	130	150	5–22–22
25	105	65	6– 3–13				

POWDERED EXTRACT GELSEMIUM.

Sample.	Chem. Assay. Per cent.	Bio. Assay. Per cent.	Date.	Sample.	Chem. Assay. Per cent.	Bio. Assay. Per cent.	Date.
1	109	125	7–28–09	10	170	194	11–11–16
2	166	250	10– 8–10	11	140	71	2– 9–17
3	111	250	11– 2–10	12	138	208	9–17–17
4	127	147	7–14–11	13	102	125	5– 6–21
5	122	312	11–17–11	14	70	156	8– 9–21
6	103	178	1–15–12	15	306	277	4–14–22
7	107	125	1–30–13	16	286	125	5–16–22
8	150	178	5– 4–14	17	85	96	12–28–22
9	171	208	7–20–15	18	100	125	4– 2–23

It will be noted from the above results that in about one-half of the samples examined, there is a parallelism between the results obtained by the two methods of assay. In the other 50% of the samples examined, however, there are marked discrepancies. As above stated, the drug contains two alkaloids, Gelsemine which is only slightly active, and Gelseminine which is a powerful poison.

The fact that the toxicity as determined by the minimum lethal dose on guinea pigs does not parallel the total alkaloidal content of the drug would indicate, therefore, that these two alkaloids are not always present in the drug in the same proportion. The determination of total alkaloid is therefore apparently not a true index to the clinical value of Gelsemium or its preparations.

Conclusion.—The results of these experiments would tend to prove, therefore, that we are without a reliable chemical means of accurately standardizing Gelsemium preparations, but that they can be standardized by physiologic means as outlined in this paper.

PHARMACODYNAMIC LABORATORIES,
H. K. MULFORD COMPANY, PHILA.,
AUGUST 16, 1923.