equivalent to $^4/_5$, $^3/_5$ or less, were then tried. The muscle would be considered satisfactory provided the heights of contractions showed a quantitative relationship with the doses employed. An example is well illustrated in Fig. 1. It must be emphasized that the height of contraction (peak) is the only criterion, and that the same dose of ergonovine makes the muscle repeat its response with exactness.

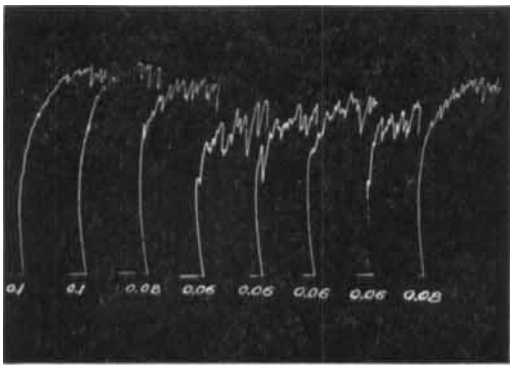

Fig. 1.—An Example of a Utilizable Strip of the Isolated Rabbit's Uterus.

The numbers indicate doses of ergonovine maleate in milligrams. Note the quantitative relationship with the amounts of the drug applied.

DISCUSSION

As shown in Table I, the results conclusively prove that subcutaneous injection of estrone or stilbestrol renders the immature rabbit's uterus more irritable, more reliable in response, and thus more utilizable in the assay of ergonovine. The optimal dose of estrone per animal is obviously in the neighborhood of 12γ, and that of stilbestrol, 9γ, each divided into 6 portions and injected in 11 days. Ovariectomy did not nullify the estrone action for 83% of the uteri was fully reactive. Although the white rabbits appeared slightly less benefited, the number of satisfactory preparations reached 80%. This is in strong contrast with the 10 control rabbits, none of which had a uterus suitable for assaying purposes. The economic advantage of the estrogenic therapy in rabbits cannot therefore, be overestimated. It is also probable that the ergonovine assay is attended by greater precision.

SUMMARY

1. Immature rabbits injected subcutaneously with divided doses of estrone or stilbestrol give rise to an overwhelmingly high percentage of uteri, which, when isolated, are suitable for ergonovine assays.

2. The optimal dose (total) of estrone is approximately 12γ per animal, and that of stilbestrol, 9γ.

3. The chief advantage of this procedure is economy. In addition, the assay may possibly be carried out with higher precision.

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Assay Methods for Phenothiazine Pharmaceuticals*

By Lewis E. Harris† and Eloise B. Kerl‡

Phenothiazine has recently assumed an important role in the veterinary pharmaceutical field since it has been found to be a very efficient anthelmintic with a comparatively low toxicity (1). To date there are no published assay methods for determination of phenothiazine purity or of phenothiazine content in pharmaceuticals. Determinations of sulfur or nitrogen by standard methods were found to be unsatisfactory and, so far, colorimetric methods have been of no value. This report describes a method for the routine control assay of phenothiazine in pharmaceutical preparations, based on the extraction and subsequent weighing of the drug. Two principal types of pheno-

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thiazine preparations are marketed—one a tablet or bolus form, the other an aqueous suspension. The following assay procedures may be used for both types of preparations.

EXPERIMENTAL

(*a*) *Tablets, Boluses or Granulations.*—Weigh and reduce to a fine powder not less than 20 tablets, 5 boluses or 10 Gm. of granulation. Weigh a sample of this powder equivalent to about 1.5 Gm. phenothiazine and transfer to fat-free filter paper. Fold the paper carefully to enclose sample and place in extraction thimble. Carry out the extraction by means of an A. S. T. M. extraction apparatus using anhydrous acetone as the solvent. The extraction should be continued about eight hours.

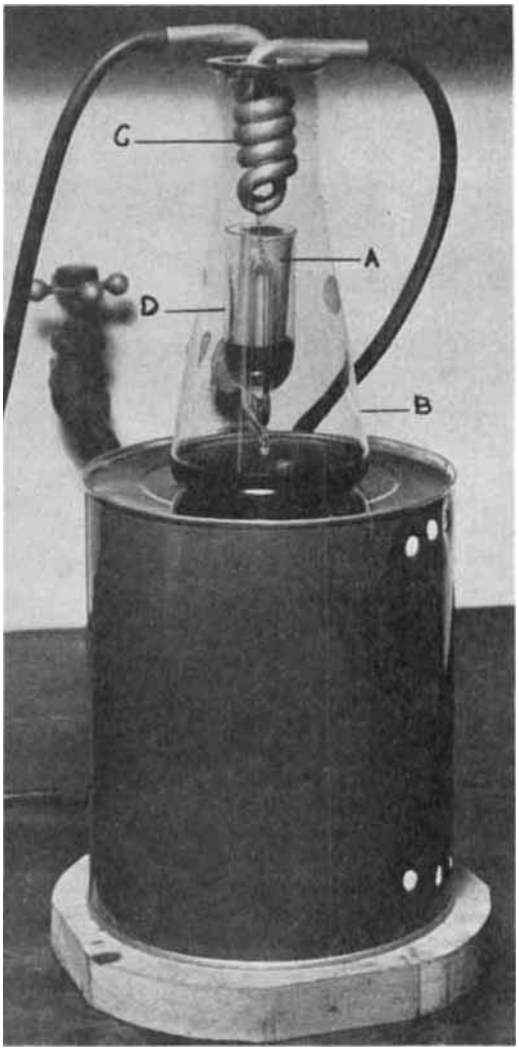

Fig. 1.—The A. S. T. M. Extraction Apparatus.
The dried material to be extracted is placed in the thimble (*a*). The acetone in the tared flask (*b*) is vaporized by low heat, condensed on the coil (*c*) and drops into the glass siphon cup (*d*).

For accuracy, the extraction flask used with the above apparatus should not weigh more than 75 Gm. After extraction is complete, the acetone is evaporated, the residue dried to constant weight and the percentage of phenothiazine calculated. In drying to constant weight, the flask should be placed in a horizontal position and the temperature kept below 80° C. to prevent loss by sublimation.

(*b*) *Suspensions.*—Agitate the sample to insure complete mixing and measure or weigh at least a 10-cc. sample. Transfer the sample to an ordinary filtering funnel fitted with a filtering cone and two sheets of fat-free filter paper of moderate retention grade (Whatman's No. 2 is satisfactory). Conduct the filtration with moderate suction and wash the residue with distilled water until all water-soluble material has been removed. Remove paper and residue, fold into a shape which will ultimately fit into the extraction thimble, place in an evaporating dish and dry at temperature under 80° C. Finally place the dried paper and residue in the extraction thimble and proceed with the extraction as directed in (*a*).

RESULTS

The results of over two hundred individual assays show this method to be satisfactory for routine control work. The tables show typical results of assays on phenothiazine suspensions, granulations, tablets and boluses.

Table I.—Phenothiazine Suspension

Sample No.	Phenothiazine Content (Grams per Fluid Ounce) Theory	Assay	Per Cent Recovery
9413	12.00	12.11	100.8
9419	12.00	11.91	99.2
9469	12.00	11.81	98.4
9968	12.00	11.99	99.9
9575	12.00	12.13	101.0
9578	12.00	12.03	100.2

Table II.—Phenothiazine Granulation

Sample No.	Phenothiazine Content Theory	Assay	Per Cent Recovery
265 L	70.50	70.51	100.0
275 L	70.50	70.25	99.6
277 L	70.50	70.96	100.6
280 L	70.50	70.71	100.3
289 L	70.50	70.68	100.2
328 L	70.50	70.94	100.6

Table III.—Phenothiazine Tablets

Sample No.	Phenothiazine Content (Grains per Tablet) Theory	Assay	Per Cent Recovery
9240	0.500	0.505	101.0
9241	0.500	0.503	100.6
9282	0.500	0.505	101.0
9355	0.500	0.497	99.4
9364	0.500	0.510	102.0
9447	0.500	0.504	100.8

Table IV.—Phenothiazine Boluses

Sample No.	Phenothiazine Content (Grams per Bolus) Theory	Assay	Per Cent Recovery
8852	12.00	12.02	100.1
9239	12.00	12.23	101.9
9288	12.00	12.16	101.3
9528	12.00	12.20	101.6
9911	12.00	12.22	101.8
9213	12.00	12.07	100.6

Each sample was run in duplicate. Results of duplicate assays show a maximum variation of ± 2.0%.

DISCUSSION AND SUMMARY

This procedure for the assay of phenothiazine, based on the extraction and subsequent weighing of the drug, was found to give good recoveries of phenothiazine from suspensions, granulations, tablets and boluses. The method, of course, would not be satisfactory if acetone-soluble substances other than phenothiazine were present in the preparation. This is not usually the case, hence this method should be useful in the majority of preparations.

REFERENCE

(1) Harwood, "Phenothiazine as an Anthelmintic," *Bulletin of U. S. D. A., B. A. I.* (September 1940).

The Utilization of Pigeons for the Biological Assay of *Adonis Vernalis*, N. F. VI*

By W. M. Benson† and L. D. Edwards‡

Since *Adonis vernalis*, N. F. VI, is a potent cardiac drug, it is contended that an assay method should be adopted for its standardization. Inasmuch as the potency of the drug cannot be determined to an accurate degree by chemical analysis, several biological procedures have been suggested. The following work deals with the application of the pigeon emetic method together with minor comparisons with the one-hour frog and cat methods in the assay of *Adonis*.

EXPERIMENTAL

Each of the three tinctures was assayed by the pigeon emetic and one-hour frog and cat methods.

The Pigeon Emetic Method.—The procedure introduced by Hanzlik (1) in 1929 was the method used in this work, although certain modifications were made. Following the suggestion of Lieb and Mulinos (2) the assay birds were previously standardized to a maximum and minimum dose of ouabain, thereby allowing the rejection of all hypo- and hypersensitive birds. The two standardization doses selected were 0.065 mg. per Kg. and 0.030 mg. per Kg. Those birds that did not emese to the larger dose and those birds which did emese to the smaller dose were rejected from use.

The "Minimum Emetic Dose" selected represents the smallest amount of *Adonis*, expressed in cc. of the tincture per Kg. of bird body weight which is capable of producing emesis in 75% of a group of eight birds within a period of 15 minutes following the injection of the drug. The results obtained are as follows:

Table I.—The Determination of the Minimum Emetic Dose of Tincture of Adonis

Dose of Tincture, Cc./Kg.	Number of Injections	Results Emesis	No Emesis
Tincture A			
0.210	8	2	6
0.215	8	7	1
0.220	8	4	4
0.225	8	5	3
0.230	8	4	4
0.235	8	4	4
0.240 (M. E. D.)	8	7	1
0.245	8	6	2
0.250	8	5	3
0.255	8	7	1
0.260	8	8	0
Tincture B			
0.165	8	2	6
0.170	8	5	3
0.175	8	4	4
0.180	8	3	5
0.185	8	1	7
0.190	8	4	4
0.195	8	4	4
0.200	8	5	3
0.205 (M. E. D.)	8	6	2
0.210	8	7	1

* Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

† An abstract of a thesis submitted to the Graduate Council of the University of Florida in partial fulfilment of the requirements for the degree of Master of Science.

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