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

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Table I.—(*Continued*)

Dose of Tincture, Cc./Kg.	Number of Injections	Results Emesis	Results No Emesis
Tincture C			
0.140	8	1	7
0.145	8	1	7
0.150	8	4	4
0.155	8	5	3
0.160	8	3	5
0.165 (M. E. D.)	8	6	2
0.170	8	6	2
0.175	8	5	3
0.180	8	6	2
0.185	8	7	1
0.190	8	7	1

The One-Hour Frog Method.—The frog procedure employed followed closely the official U. S. P. XI method for the assay of digitalis except that the frogs were standardized to ouabain, having a M. S. D. of 0.0005 mg. per Gm. of frog.

The "Minimum Systolic Dose" as herein used is defined as the smallest quantity of drug, expressed in cc. of tincture per Gm. of frog weight which is capable of producing systolic stoppage of the ventricle in 75% of a group of four frogs, at exactly one hour following the injection. The results obtained are given in Table II.

Table II.—The Determination of the Minimum Systolic Dose of Tincture of Adonis

Cc. of Tincture per Gm. of Frog	Number of Injections	Results S. S.	Results Beats
Tincture A			
0.0020	4	0	4
0.0025	4	1	3
0.0030 (M. S. D.)	4	4	0
0.0035	4	4	0
Tincture B			
0.0015	4	0	4
0.0020	4	1	3
0.0025	4	2	2
0.0030 (M. S. D.)	4	3	1
0.0035	4	4	0
Tincture C			
0.0015	4	1	3
0.0020	4	0	4
0.0025 (M. S. D.)	4	3	1
0.0030	4	3	1
0.0035	4	4	0

The Cat Method.—The method used was much like that originally described by McGuigan and McGuigan (3) using cats instead of dogs. In this method of assay the anesthetic used was sodium pentobarbital, 35 mg. per Kg. Artificial respiration was maintained throughout the assay period. The tinctures were administered by femoral injection at the rate of 0.1 cc. per Kg. of cat weight every five minutes until death. The average fatal doses for the three tinctures are given in Table III.

Table III.—The Determination of the Minimum Fatal Dose of Tincture of Adonis

Weight of Cat, Kg.	Number of Injections, 0.1 Cc./Kg.	Fatal Dose, Cc. Tr./Kg.	Average Fatal Dose, Cc. Tr./Kg.
Tincture A			
1.92	11	1.1	
2.10	14	1.4	1.20
2.18	11	1.1	
Tincture B			
2.95	11	1.1	
2.55	10	1.0	1.10
2.20	12	1.2	
Tincture C			
3.90	11	1.1	
2.35	11	1.1	1.10
1.55	11	1.1	

Discussion of Results.—Arbitrarily selecting Tincture of Adonis (B) as 100% in strength, the per cent deviation in potency of the three tinctures has been determined when assayed by the three different methods as outlined (see Table IV).

Table IV.—Estimation of the Per Cent Potency and Deviation of Tinctures of Adonis (A), (B) and (C) When Assayed by Three Different Methods. Tincture B is Arbitrarily Selected as Being 100% in Strength

	Tincture	Cc. Tr./Kg.	Per Cent Potency	Per Cent Deviation
Frog method, M. S. D.	A	3.00	100.0	0.0
	B	3.00	100.0	0.0
	C	2.50	120.0	20.0
Cat method, M. F. D.	A	1.20	91.6	8.4
	B	1.10	100.0	0.0
	C	1.10	100.0	0.0
Pigeon method, M. E. D.	A	0.225	85.3	14.7
	B	0.190	100.0	0.0
	C	0.165	115.0	15.0

Theoretically, the per cent deviation from the standard should be the same for the several assay methods. In the comparison drawn here, the per cent potency of Tincture of Adonis (A) assays 100% by the frog method, 91.6% by the cat method, and 85.3% by the pigeon emetic method. Tincture C assays 120% by the frog method, 100% by the cat method and 115% by the pigeon method. Although the potency varies somewhat with the method of assay the percentage strengths run fairly parallel. In no case has the extrene per cent of deviation been greater than 20%.

In the pigeon method when birds were given only short rest periods between injections a conditioning effect was observed.

These results, however, indicate that the pigeon emetic method is suitable for the assay of *Adonis vernalis*.

CONCLUSIONS

1. The results of the assay of tinctures of *Adonis* by the pigeon emetic method parallel the results obtained by the one-hour frog and fatal cat methods.

2. Because the pigeon method possesses a definite end-point, is simple, economical and reasonably accurate, its use for the assay of *Adonis vernalis* appears worthy of consideration for adoption in the National Formulary as the official method.

3. Inasmuch as the standardization of the birds to a maximum and minimum dose of ouabain seems important, the recommendation is made that this procedure be carried out prior to the assay of the drug. It is further suggested that a rest period of one month be allowed between successive injections.

REFERENCES

(1) Hanzlik, P. J., *J. Pharmacol.*, 35 (1929), 363.

(2) Lieb, C. C., and Mulinos, M. G., *Ibid.*, 48 (1933), 282.

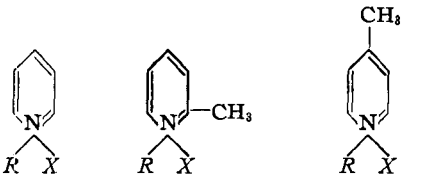
(3) McGuigan, R. A., and McGuigan, H. A., *Ibid.*, 63 (1938), 76.

Germicidal Activity of Some Quaternary Ammonium Salts*

By H. G. Kolloff, A. P. Wyss, R. E. Himelick and F. Mantele

Following the report of the germicidal activity of quaternary ammonium salts by Domagk in 1935 (1), considerable attention has been directed toward compounds of this type.

Since the introduction of a methyl group into the phenol molecule increases the germicidal activity (2, 3), it was thought desirable to compare the activity of certain alkyl pyridinium halides with the corresponding alpha- and gamma-picolinium compounds. The chemical relationship of these quaternary salts may be represented as follows:



Pyridinium alkyl halides | α-Picolinium alkyl halides | γ-Picolinium alkyl halides

in which R = $C_{12}H_{25}$-, $C_{14}H_{29}$- or $C_{16}H_{33}$-, and X = halogen.

The method used in the preparation of the 27 compounds herein reported was essentially that reported by Knight and Shaw (4).

Preliminary germicidal tests were made according to the F. D. A. method using *Staphylococcus aureus* at 37° C. (5). Each test was accompanied by a special control to be certain that the dilution reported was germicidal. The preliminary results indicate that the introduction of a methyl group into the aromatic nucleus of these alkyl pyridinium halides does not in general increase the germicidal activity.

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EXPERIMENTAL

Materials:

Pyridine—Mallinckrodt, analytical reagent.

α-Picoline—Eastman Kodak Co., redistilled: b. p., 126–127.5° C.; n_D^{20}, 1.5009.

γ-Picoline—Pure γ-picoline was prepared by the procedure recently described by Kolloff and Hunter (6); b. p., 142° C.; n_D^{20}, 1.5052.

Tetradecyl chloride (b. p. 126–129° C. at 1.5 mm.; n_D^{20}, 1.4465) and

Hexadecyl chloride (b. p. 120–124° C. at 0.07 mm.; n_D^{20}, 1.4501) were prepared by the method of Norris and Taylor (7).

Dodecyl iodide (b. p. 142–144° C. at 5.0 mm.; yield, 32.6 Gm.) and

Tetradecyl iodide (b. p. 178–181° C. at 13.0 mm.; yield, 31.5 Gm.) were obtained from 25.0 Gm. quantities of the corresponding alcohols using the method of Levene and West (8).

All other alkyl halides were obtained from the Eastman Kodak Company and were used as supplied without further purification.

Preparation of the Quaternary Ammonium Salts.—The procedure was similar to that used by Knight and Shaw (4), and the following description for the preparation of dodecyl α-picolinium iodide serves as an illustration:

Dodecyl α-Picolinium Iodide.—Three grams of dodecyl iodide and 1.0 Gm. of α-picoline were mixed