as much water (initial analysis) as the specimens opened and assayed immediately after purchase. The specimen which had been opened weekly in Minneapolis for 8 weeks (but not analyzed there) (F) contained 12.1 per cent of water after its return to the Washington laboratory, or nearly 6 molecules. After monthly opening and sampling in Washington it became stable in 10 months after opening, or 1 year after purchase. Likewise, the specimen which had been opened in Denver (G) contained 12.26 per cent of water (or nearly 6 molecules) after its return to Washington. It became stable in 12 months after opening or 14 months after purchase.

TABLE III.—WATER CONTENT OF QUININE SULPHATE AFTER STORAGE FOR DIFFERENT PERIODS.

Sample.	Treatment before Initial Analysis, Per Cent.	Water Content Initial Analysis, Per Cent.	Water Content 1 Mo. after Opening, Per Cent.	Water Content 2 Mos. after Opening, Per Cent.	Water Content 4 Mos. after Opening, Per Cent.	Water Content 6 Mos. after Opening, Per Cent.	Water Content 8 Mos. after Opening, Per Cent.	Water Content 12 Mos. after Opening, Per Cent.
A	Opened immediately	12.91 12.91 13.18	8.93	6.98	No assay	No assay	No assay	4.84
B	Opened immediately	13.48 13.44	9.56	6.40	5.00	No assay	No assay	4.51
C	Stored 1 month after purchase	13.53 13.00	12.65 11.84	8.70 8.45	4.56	4.62	No assay	4.67
D	Stored 3 months after purchase	12.09 11.88	10.33	8.51	7.36	7.21	6.80	4.86
E	Stored 4 months after purchase	11.25 11.21	9.78	8.95	7.44	7.44	5.25	4.58
F	Opened weekly for 2 mos. but no samples taken, then opened monthly for analysis	12.10 12.09	11.54 11.53	10.42	8.07	6.58	No assay	4.74
G	Opened weekly for 2 mos. but no samples taken, then opened monthly for analysis	13.27 11.25	9.13 9.06	8.78 8.20	7.94	No assay	No assay	4.81

These experiments show that during storage in a climate comparable to Washington, D. C., crystallized quinine sulphate progressively loses water of crystallization until it contains about 2 molecules (4.60%) after which it remains practically stable. The more frequently the specimen is opened the more rapid is the loss. Specimens which are opened occasionally but which are not otherwise disturbed, such as the packages sent to Minneapolis and Denver, do not lose their water of crystallization very rapidly. The conditions under which Specimens A and B were kept probably represent those obtaining in drug store practice more nearly than is the case with the other specimens.

THE HYDRATION OF EMETINE HYDROCHLORIDE AND CODEINE PHOSPHATE.*

BY H. WALES.[1]

I. Emetine Hydrochloride.—The statement in the tenth edition of the United States Pharmacopœia that emetine hydrochloride "contains variable amounts of water of crystallization" is the obvious conclusion to be drawn from the reports of those who have published data on this product.

Paul and Crownley (1) state that considerable difficulty was encountered in obtaining the salt in a state fit for analysis on account of the large quantity of

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[1] Drug Control, Food and Drug Administration.

mother liquor retained by the crystals, and decide that it contains six molecules of water of crystallization. Hesse (2) states that emetine hydrochloride crystallizes from water with eight molecules of water of crystallization; that four molecules are easily given up on exposure to the air, and that the anhydrous salt will readily take up $4H_2O$ on exposure to the air. He computes the data given by Frerichs and de Fuentis Tapis (3) as showing $4H_2O$ for a commercial sample and $8H_2O$ for one which they obtained by adding ether to an alcoholic solution of the salt. Hesse states also that Keller (4) found nearly $4H_2O$ in a commercial sample and $3H_2O$ in one which was obtained by adding ether to an alcoholic solution. Carr and Pyman (5) state that emetine hydrochloride recrystallized from water and "dried in air until of practically constant weight retains $7H_2O$." They claim that when recrystallized from methyl alcohol it contains $3^1/_2H_2O$. As indicated by Table I the same lack of consistency is shown in the standards provided by the various pharmacopœias for the water content of emetine hydrochloride. However, only three of these pharmacopœias venture to ascribe a definite hydrate to the product. The British and Brazilian Pharmacopœias state that it contains $7H_2O$ and the Swiss Pharmacopœia that it contains "about $4H_2O$." Incidentally the formula of $C_{30}H_{44}O_4N_2.2HCl$ given in the Pharmacopœias of the United States and Brazil is not as well authenticated as that of $C_{29}H_{40}O_4N_2.2HCl$ which is used by Netherlands, Great Britian, Switzerland and Denmark (5), (7).

TABLE I.—MOISTURE STANDARDS FOR EMETINE HYDROCHLORIDE.

	Min.	Max.
Pharmacopœia of Japan (1919)	..	15%
United States Pharmacopœia (1925)	..	19%
Deutsches Arzneibuch (1926)	..	10%
Nederlandsche Pharmacopee (1926)	10%	14%
Pharmacopœia Brazil (1929)	..	19%
British Pharmacopœia (1932)	15%	19%
Pharmacopœia Helvetica (1933)	10%	13%
Pharmacopœia Danica (1933)	..	15%

Coormans (6) calls attention to the variation in water content of emetine hydrochloride as provided by the different European pharmacopœias and finds that samples manufactured in different countries meet the pharmacopœial requirements of the country of manufacture. The actual amount of water found in the samples of emetine hydrochloride examined by the several investigators is shown in the following table.

TABLE II.—WATER CONTENT OF EMETINE HYDROCHLORIDE.

	Per Cent.	Moles.
Paul and Crownley	19.59	7.5
Frerichs and de Fuentis Tapis	12.57	4.4
	19.51	7.4
Keller	10.16	3.5
	8.31	2.8
Hesse	19.91	8.2
	12.73	4.5
	12.54	4.4

TABLE III.—VAPOR PRESSURE AT 25° C. OF EMETINE HYDROCHLORIDE CONTAINING VARYING AMOUNTS OF WATER.

Water in Sample, Per Cent.	Moles Water in Sample.	Vapor Pressure in Mm.
28.14	12.0	23.0
26.84	11.3	22.1
25.62	10.8	21.8
24.29	9.9	20.0
22.04	9.1	18.4
19.93	7.6	16.0
18.65	7.0	14.5
17.56	6.6	13.1
16.37	6.0	11.5

TABLE II.—*Continued.*

Carr and Pyman	17.4	6.5
	19.0	7.3
	18.2	6.9
	18.4	7.0
	17.3	6.5
	19.2	7.3
Coormans	16.34	6.0
	13.0	4.6
	15.0	5.4
	13.9	5.0
	10.25	3.5
	9.5	3.2

TABLE III.—*Continued.*

15.24	5.5	10.0
12.69	4.5	7.0
10.62	3.7	4.6
8.51	2.8	3.3
6.97	2.3	2.5
4.90	1.6	1.2
3.19	1.0	0.8
0.83	0.3	0.2

The vapor pressure of emetine hydrochloride, progressively dehydrated at 25° C., was determined by the method already described for quinine sulphate (8). As can be readily seen from the accompanying table and curve no evidence of a hydrate is shown. The vapor pressure of the solution of water in emetine hydrochloride is so low that the product will be in equilibrium with atmospheric conditions when it contains between 8% and 16% of water. This water is, however, merely adsorbed or dissolved in the emetine hydrochloride. It does not exist in the form of a hydrate.

II. Codeine Phosphate.—Codeine phosphate is described in the British Pharmacopœia (1932) as containing one molecule of water of crystallization. The German (1926), Swiss (1933) and Danish (1933) Pharmacopœias state that it contains one and one-half molecules of water while the United States (1926), Swedish (1925), French (1925) and Brazilian (1929) Pharmacopœias ascribe two molecules of water of crystallization to the product.

Anderson (9) reports that codeine phosphate crystallized from water contains approximately $1^1/_2H_2O$. Schmidt (10) states that when crystallized from water it contains $2H_2O$ and from dilute alcohol $^1/_2H_2O$. Beilstein (11) and Tambach and Henke (14) both give the formula with $1^1/_2H_2O$. Schaefer (12) states that "all the preparations on the market contain only $^1/_2$ molecule of water of crystallization. The salt with 2 molecules of water exists but it is a practical impossibility to produce it for commercial purposes." Henry (13) states that codeine phosphate contains 1, $1^1/_2$ or $2H_2O$.

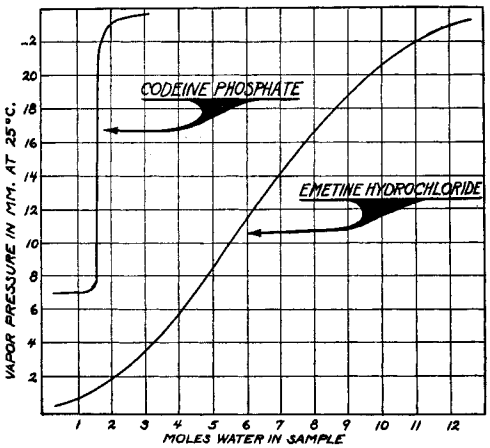

Fig. 1.

Codeine phosphate progressively dehydrated at 25° C. by the method described above gave the following results.

As will be seen from the accompanying curve codeine phosphate crystallized

from water at room temperature contains one and one-half molecules of water of crystallization. No evidence of any other hydrate is indicated by these results.

TABLE IV.

Per Cent Water in Sample.	Moles Water in Sample.	Vapor Pressure in Mm.	Per Cent Water in Sample.	Moles Water in Sample.	Vapor Pressure in Mm.
8.15	1.96	23.0	4.56	1.05	7.0
7.61	1.82	21.0	3.71	0.85	7.0
6.80	1.61	18.0	3.20	0.73	6.6
6.57	1.55	9.0	2.96	0.67	7.0
6.22	1.47	7.5	2.07	0.47	6.5
6.06	1.42	7.1	1.49	0.33	6.0
5.53	1.24	7.0	1.18	0.26	5.0
4.84	1.12	7.0			

REFERENCES.

(1) Paul and Crownley, *The Pharmaceutical Journal and Transactions*, 25, iii (1894), 373.
(2) Hesse, *Annalen*, 405 (1914), 1.
(3) Frerichs and de Fuentis Tapis, *Archiv der Pharmazie*, 240 (1902), 390.
(4) Keller, *Ibid.*, 249 (1911), 519.
(5) Carr and Pyman, *J. Chem. Soc.*, 105 (1914), 1602.
(6) Coormans, *J. pharm. Belg.*, 14 (1932), 389.
(7) Pyman and Brindley, *Chem. and Ind.*, 46 (1927), 356.
(8) Wales, JOUR. A. PH. A., 23 (1934), 793.
(9) Anderson, *Annalen*, 77 (1851), 353.
(10) Schmidt, *Apoth. Ztg.*, 5 (1890), 366; through *Am. J. Pharm.*, 62 (1890), 444.
(11) Beilstein, "Organische Chemie," 3, 902 (1897 Edition).
(12) Schaefer, *Am. J. Pharm.*, 82 (1910), 220.
(13) Henry, "Plant Alkaloids" (1924), 263.
(14) Tambach and Henke, *Pharm. Zentralhalle*, 38 (1897), 159; through *Chem. Centralblatt*, 68 (1897), 947.

THE BIOASSAY OF THE ANTERIOR PITUITARY-LIKE SEX HORMONE (ANTUITRIN S).*

BY L. W. ROWE, A. SIMOND AND W. O. NELSON.

Although several bioassay methods for evaluating the anterior pituitary-like sex hormone from pregnancy urine have been suggested, none of them has been subjected to adequate critical scrutiny for accuracy nor has there been much basis for a comparison of values obtained by different methods. It will be the purpose of this article to present in detail the method which has been used to control accurately the potency of a commercial product (Antuitrin S) as well as to give data comparing activities obtained in the assay of the same preparation by several other recognized methods.

Zondek and Aschheim (1) suggested the use of baby female white mice weighing 6 to 8 Gm., for assaying anterior pituitary sex hormone preparations.

In 1931, Fevold, Hisaw and Leonard (2) published a method for the assay of the anterior pituitary sex hormone using immature female white rats 20 to 25 days old as the test animals. Later, Fevold, *et al.* (3) used 21- to 23-day old rats and the same technique but they also reported that rabbits 12 weeks old were even more

* Scientific Section, A. PH. A., Washington meeting, 1934.