

17	Non-Polarized Macht-Pfund 1 hour T° 25° C.	Control Apparatus.	2 Sterile	2 Sterile	2 Sterile	2 Sterile	2 Sterile	2 Grew
18	Polarized Light. Pfund Apparatus. T° 25° C.	Macht- 4 hours.	1 Sterile				2 Sterile	1 Grew
19	Non-Polarized Macht-Pfund 4 hours T° 25° C.	Control Apparatus.	2 Sterile				2 Sterile	1 Grew
20	Dilution Controls.							4 Grew

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THE COLORIMETRIC ASSAY OF STROPHANTHUS.*

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Although a complete account of the application by Knudson and Dresbach (1) and (2) of the Baljet reaction (3) to the colorimetric testing of strophanthus preparations was apparently never published, it was shown in the short abstract available, that the same technique was used as in testing digitalis preparations; that ouabain was used as a standard; and that comparison with the Hatcher Cat Method was entirely satisfactory from the standpoint of accuracy.

In spite of unsuccessful results with the colorimetric assay of digitalis preparations, (4) the application of the method to strophanthus seemed promising since such dilute solutions could be used that it would be unnecessary to subject them to the purifying process. Also ouabain or Tr. Strophanthus should be suitable as standards. Accordingly short series of tests were made on the same preparations by both the colorimetric and the Houghton frog methods. Since the drug differs from digitalis even though closely related in pharmacological and therapeutic action and since the results obtained were somewhat more favorable it was considered advisable to make a separate report of these tests rather than include them in the more extended series of digitalis tests.

TABLE I.

Preparation.	Standard.	Color test.	Frog test.	Color error.
Ouabain A	Ouabain X	100%	73%	27% high
Ouabain B	•Ouabain X	78%	61%	22% high
Ouabain C	Ouabain X	140%	61%	56% high
Ouabain C	Ouabain X	100%	61%	39% high
Ouabain X (U. S. P. X)	Digitalin	100%	73%	27% high
Tr. Stroph. 1890	Ouabain X	50%	50%	
Tr. Stroph. 781,111	Digitalin	215%	155%	28% high

In this series four different lots of ouabain were compared by the two methods, a 1 to 25,000 solution being used in the color tests and no purification made. In each color test of ouabain "C" the result was very high and the two results did not agree at all closely. However, this was the first series and later comparisons were more satisfactory. It was found in this series that a U. S. P. 1890 tincture,

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which was being used as a standard in the frog tests, was equivalent to the 1-25,000 solution of standard ouabain X when diluted 1 to 70.

TABLE II.

Preparation.	Standard.	Color test.	Frog test.	Color error.
Tr. Stroph. 781,642	Tr. S. 1890	100%	120%	20% low
Tr. Stroph. 770,265	Tr. S. 1890	100%	85%	15% high
Strophanthone 073,178	Tr. S. 1890	200%	120%	40% high
Strophanthone 073,178	Tr. S. 1890	155%	130%	16% high
Tr. Stroph. 774,382	Tr. S. 1890	135%	120%	11% high
Strophanthone 777,962	Tr. S. 1890	125%	110%	12% high
Strophanthone 076,234	Tr. S. 1890	90%	90%	
Tr. Stroph. 160,771	Tr. S. 1890	100%	110%	10% low
Strophanthone 076,234	Tr. S. 1890	120%	120%	
Tr. Stroph. 784,289	Tr. S. 1890	100%	100%	
Tr. Stroph. 784,777	Tr. S. 1890	114%	110%	4% high
Tr. Stroph. 134,288	Tr. S. 1890	143%	145%	
Tr. Stroph. 784,860	Tr. S. 1890	150%	155%	3% low
Strophanthin 179,932	Tr. S. 1890	144%	130%	10% high

In this second series eight tinctures, five purified aqueous preparations and one Kombe strophanthin were tested using the frog test standard tincture strophanthus U. S. P. 1890 diluted 1 to 70 as the color standard. The extremes ranged from 20% low to 40% high with an average error of 10%. If the two extremes are eliminated the average error is only 6.7%. The later results in this series are fairly satisfactory showing that in the case of strophanthus where total activity and alimentary absorbable activity are probably proportional though not equal and where a purification method is not necessary the color method can be used as a preliminary test. This result should be checked with a small number of frogs but the time and expense of an assay would be appreciably decreased without sacrificing accuracy.

As a further proof of the color method a series of preparations previously tested by each method were diluted and subjected to tests, the dilutions being unknown to the one making the tests. In most of these cases the frog method was not applied since its accuracy within a reasonable experimental error had been previously established.

TABLE III.

Preparation.	Standard.	Color test.	Actual.	Color error.
From Tr. 784,289	Tr. S. 1890	75%	67%	10% high
From 076,234	Tr. S. 1890	40%	31%	29% high
From Tr. 784,289	Tr. S. 1890	75%	80%	7% low
From Tr. 784,289	Tr. S. 1890	25%	29%	16% low
From Tr. 3 numbers	Tr. S. 1890	286%	293%	2% low
From Tr. 3 numbers	Tr. S. 1890	71%	64%	10% high
From Ouabain X	Ouabain	33%	31%	6% high
From Tr. U. S. P. 1890	Ouabain	26%	25%	4% high

The average variation in this series of eight unknowns is 10.5% while if the one aqueous preparation is eliminated the average variation is 8%. There was such a fair average agreement between the two methods in this series of unknowns and in the later tests of regular lots, it was decided that the colorimetric method

was satisfactory for use as a preliminary to a short final check of the activity of strophanthus preparations by the M. L. D. Frog Heart Method.

Trials were made on one lot of F. E. Squill, one of F. E. Convallaria and one of F. E. Veratrum Viride all of which can be tested by the M. L. D. Frog Method. The purified Veratrum Viride failed to develop any color with the alkaline picrate. The purified squill solution developed color very slowly and then not enough color in proportion to its physiological activity. The purified convallaria solution also failed to develop color in proportion to its physiological activity compared with digitalis.

SUMMARY.

1. Strophanthus preparations can be assayed in a preliminary way and with fair accuracy by the picric acid colorimetric method—the comparison with results by the frog method being reasonably satisfactory.

2. Either U. S. P. ouabain, 1 to 25,000 or tincture strophanthus U. S. P. X, 1 to 140 are quite suitable as standards.

3. Purification of solutions is not necessary and total activity is apparently always proportional though not equal to alimentary absorbable activity.

4. The colorimetric method is not suitable for the assay of squill, convallaria and veratrum preparations as judged by one attempt on each.

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- (1) Knudson and Dresbach, *Proc. Soc. Exptl. Biol. Med.*, 19, 389 (1922).
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- (3) H. Baljet, *Schweiz. Apoth. Ztg.*, 56, 71 and 84 (1918).
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STABILITY OF TENTH NORMAL POTASSIUM DICHROMATE VOLU- METRIC SOLUTION.

BY M. W. CAREY.

"I found an old bottle of Potassium Dichromate tenth normal V. S. recently in our laboratory stock which had been made and standardized in 1903. At that time it had a factor of 1.004.

"Since the solution appeared to be in good condition I refactored it and found rather to my surprise that there had been no appreciable change in strength. The factor as I find it now is 1.001.

"The solution had been kept during the past 23 years in an 8-liter, flint glass bottle with an ordinary cork stopper—the bottle about two-thirds full. In color and clarity it appears like a freshly-made solution, on agitation, however, a slight sediment was disclosed in the bottom of the bottle.

"It is surprising that a solution of this kind should keep so well."

ANALYTICAL LABORATORIES,
E. R. SQUIBB AND SONS.