4. Thallium may be isolated from various structures of the body even when death is delayed for thirteen days.

5. Spectroscopic methods of analysis are much more sensitive than chemical ones.

6. Close supervision should be kept of all thallium used in rodent poisoning, and it should be employed only by trained men.

June 23, 1931.

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A STUDY OF THE BORNTRAEGER COLOR REACTION AND THERAPEUTIC ACTIVITY OF CASCARA SAGRADA.*

BY S. W. MORRISON.[1]

There is no satisfactory method to standardize or assay cascara preparations. As a consequence there is some variation in the therapeutic activity of the extracts on the market.

Considerable work has been done by Eaton (1), Fuller (2) and others in an effort to isolate the active ingredients, and to establish a reliable means of comparing the relative activity of different cascara products.

The Borntraeger reaction has been advocated as a means of standardizing fluidextract of cascara and has been utilized by Peter Valaer (3) and others (4) to make quantitative tests for cascara.

The Borntraeger test consists in extracting the ether-soluble constituents from the cascara preparation and developing a red color in the yellow ether solution by the addition of ammonia water. The depth of the colors is measured in a Lovibond tintometer against a suitable standard color.

Various solvents and methods have been used by Nitardy (5), and Milford Harris and Davy (6) to extract the active portion from the cascara bark. Attempts have also been made to obtain a potent preparation of cascara free from bitter taste and griping action.

It has been found also that cascara products vary greatly in the degree and shade of color produced with the Borntraeger test.

The object of the present investigation was to determine if the color tests for cascara sagrada parallel its therapeutic activity.

EXPERIMENT I.

Two hundred and fifty grams of ground cascara bark were extracted in a percolator with boiling water as directed in the U. S. P. X. The percolator was surrounded by a hot water jacket to retain the heat during the extraction.

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Seven successive two-liter percolates were collected and evaporated on a water-bath to a volume shown in Table I, and alcohol then was added as a preservative. Following this, two four-liter portions were collected and concentrated. Percolation was stopped at this point as the percolate had only a pale straw color and did not darken on the addition of ammonia water (see Table I).

After the nine extractions (A) Table I, forty Gm. of the exhausted drug were boiled under a reflux condenser in a liter of 2% sulphuric acid for 90 minutes, neutralized with sodium hydroxide, concentrated on a water-bath and then extracted with ether and alcohol to separate the cascara extractive from the sodium sulphate. The ether-alcohol solution was evaporated to dryness and redissolved in 100 cc. of water. (No. 10, Table I.)

Another 40-Gm. portion (B) was boiled in a liter of water for one hour, filtered, and the filtrate evaporated to 100 cc. (No. 11 in Table I.)

Twenty grams of the exhausted drug B were then boiled with 500 cc. of 2% sulphuric acid for one hour, neutralized, filtered, extracted with alcohol, evaporated to dryness and redissolved in 50 cc. of water. (No. 12, Table I.)

The remainder of portion B (20 Gm.) was boiled with 500 cc. of water for one hour, filtered and concentrated to 50 cc. (No. 12A, Table I.)

The amount of color in each of these extracts was determined by using the tintometer, taking 2.2 as the yellow standard and 1.1 as the red standard for a U. S. P. fluidextract, following the method described by Peter Valaer (3).

TABLE I.—EXPERIMENT I.

A. Successive portions.	I. Liters percolate.	II. Cc. of concentrate.	III. % Strength. Yellow.	IV. Red.	V. Average %.	VI. Equiv. cc. U. S. P. fluidext.
1	2	160	126	131	128	208
2	2	40	30	34	32	12.8
3	2	40	31	30	30.5	12.2
4	2	40	14	16	15	6.0
5	2	30	19	18	18.5	5.5
6	2	20	10	10.5	10.2	2.1
7	2	20	13	16	14.5	2.9
8	4	20	8.8	8.7	8.75	1.7
9	4	20	8.8	9.2	9	1.8
10 acid decoct.	6.2	625	1.3	1.7	1.5	9.3
11 aq. decoct.	6.2	625	1.3	2.0	1.65	10.2
12 aq. decoct.	6.2	625	1.0	1.5	1.25	3.9
12A acid decoct.	6.2	625	1.1	1.8	1.45	4.5

NOTE: In portions 10, 11, 12 and 12-A the figures in Columns I and II represent the amount of percolate and concentrate that would have been obtained if the entire amount of original drug (250 Gm.) had been used.

The variations in the strengths of the percolates are probably due to the difference in time of extraction which sometimes resulted in a rise of strength higher in a later than in a preceding portion. Variations in the rate of percolation and the temperature of the water may also account for the slight irregularity. The concentration selected after evaporation was such that all the extractive matter would be held in solution and that the therapeutic activity might more nearly approach that of a fluidextract.

For the therapeutic tests, human subjects were used. They were placed on a normal diet which was not varied during the tests. Care was taken to make all tests at the same time of day and under exactly the same conditions.

TABLE II.—EXPERIMENT I.

The following table gives a condensed account of the therapeutic experiment.

Portions of percolate in order.	Color test % strength.	Dose cc. of sol. taken for therap. test.	Approx. equiv. of U. S. P. dose taken.	Therapeutic effect.	Date.
1	128	1.6	2	100% positive	2/7/31
	128	1.0	1.3	90 mild	2/20/31
	128	0.8	1.0	75	1/28/31
2	32	6.6	2	0	1/24/31
3	30.5	15	5	20 ?	1/10/31
4	15	13	2	0	1/8/31
5	18.5	22	4	10	1/19/31
6	10.2	29	3	0	
7	14.5	35	5	0	1/31/31
8	8.75	35	3	0	1/22/31
9	9	60	5	0	1/31/31

With percolate No. 1 the color index corresponded quite closely with the therapeutic effect. In all other following percolates, however, the therapeutic effect fell rapidly and with 100% of color no therapeutic effect was elicited.

In No. 4, corresponding to 200% color index, there was no therapeutic effect.

In No. 7, corresponding to 500% color index, there was no therapeutic effect.

In No. 9, corresponding to 500% color index, there was no therapeutic effect.

These are outstanding examples to which the others conform in varying degrees.

In No. 4, for example, two times the therapeutic dose was taken, this corresponds to twice the color of one therapeutic dose or 200%. In No. 9, five times the U. S. P. therapeutic dose was taken which corresponds to 5 times the color index.

EXPERIMENT II.

A 500-Gm. sample of powdered cascara bark was extracted (percolated) with 11 liters of boiling water and the percolate concentrated to 500 cc. Neither the Borntraeger reaction nor the therapeutic activity of this concentrate was determined since in Experiment I, a similar product possessed 100% activity. Subse-

TABLE III.

Successive portions.	Liters of percolate.	Concen. extract.	Equiv. U. S. P. dose.***	Equiv. of U. S. P. doses taken.	Therapeutic effect.*
1	11	500 cc.	Not tested	Not tested	Not tested
2	2	1.85 Gm.	0.3 Gm.	11	90% mild
3	4	3.43 Gm.	0.4 Gm.		
4	8	2.05 Gm.	0.7 Gm.		
5	.8	2.00 Gm.	0.8 Gm.	8.5	0
**6 boiled	3	lost			
**7 boiled	2	1.6 Gm.	1.1 Gm.		

* The therapeutic effect given represents the average effect on all the subjects and not individual effects.

** In 6 and 7, the cascara was boiled with water, not subjected to percolation.

*** Amount equivalent, as determined by Borntraeger reaction, to one U. S. P. dose.

quent percolates as shown in Table III were evaporated to dryness; 0.5-Gm. samples of each dry extract were dissolved separately, each in 10 cc. of water and assayed for color index and therapeutic activity.

The results are tabulated on page 1278.

According to Table III, Nos. 2 and 3 combined, 1100% of color index gave 90% therapeutic effect.

Nos. 4, 5 and 7 combined, 850% color index gave no therapeutic effect. These tests show conclusively that cascara preparations may be prepared in which there is no relation between the color test and the therapeutic activity.

EXPERIMENT III.

The effect of different organic solvents on cascara bark was studied in an effort to determine the best solvent that will produce a fluidextract free from color and bitterness and still therapeutically active.

Powdered cascara bark was macerated three days with the organic solvent and the resulting solution concentrated to the strength of a fluidextract. The marc was then dried and percolated with boiling water by the U. S. P. method and a fluidextract prepared. The taste, color test and therapeutic activity were determined on each portion.

TABLE IV.

1st extraction.	Taste very bitter.	Color test.	Therap. activity.	2nd Extraction with boiling water.	Taste slightly bitter.	Color test.	Therap. activity.
		%	%			%	%
Alcohol		93	20	The marc		68	100
Acetone		63	20	from each		68	80
Ethyl Acetate		72y 38r	0	of the preceding, was		68	100
Chloroform		18	0	extracted		95	100
Ether		15	0	with water.		97	80
Benzene		9	0			100	90
Toluene		8	0			100	100
Benzine		4	0			107	75

NOTE: Regarding the color test: It is possible in some cases to obtain a color test of over 100. For example if one takes 100 Gm. and extracts it with a solvent, all of the color may pass into the solvent and all of the therapeutic activity remain in the marc, or again the solvent may extract more color than does water.

Alcohol, acetone and ethyl acetate apparently extract a therapeutically inert coloring principle as all the fluidextracts assay the same (68%) by color, but retain practically all their therapeutic activity.

EXPERIMENT IV.

A 50-Gm. sample of ground cascara bark was thoroughly extracted with five successive solvents, as follows: Benzine, ether, acetone, ethyl acetate, alcohol, and finally with boiling water. Acetone and alcohol extracted the most color. The fluidextract prepared from the marc with boiling water was only slightly bitter and assayed 13% by the color test. When administered to four different persons, it was found to be 100% therapeutically active.

EXPERIMENT V.

Alcohol (95%) was again used to extract ground cascara bark. Percolation was continued over a period of three weeks before the percolate was quite free from color. The alcoholic percolate was concentrated to represent 1 Gm. of bark per cc. The color test indicated a preparation 170 % as compared to the U. S. P. fluidextract. The therapeutic activity was about 40% of the U. S. P. fluidextract.

The bark was then exhausted with boiling water and a fluidextract prepared. The color test was 4%. The therapeutic activity was approximately 60%. The aqueous fluidextract thus prepared had only a slight bitterness.

EXPERIMENT VI.

A sample of cascara bark was percolated for three weeks with acetone. The acetone percolate was reduced to a volume corresponding to a fluidextract. The marc was then extracted with boiling water and an aqueous fluidextract prepared.

The acetone fluidextract assayed 131% by the color test and the therapeutic activity was estimated to be 75% to 90%. The aqueous fluidextract assayed 16% and had a therapeutic activity of about 50%.

Following table shows color and therapeutic results of Experiments IV, V and VI.

TABLE V.

1st extraction.	Taste bitter.	Color test.	Therap. activity.	2nd extraction.	Taste slightly bitter.	Color.	Therap. activity.
Mixture of organic solvents		Not tested	Not tested	With		13%	100%
Alcohol 3 weeks		170%	40%	boiling		4%	60%
Acetone 3 weeks		131%	75 to 90%	water		16%	50%

EXPERIMENT VII.

An attempt was next made to separate the active ingredients of cascara by salting out with different inorganic salts. The method of Diefenbach (7) was used. The fluidextract was evaporated to remove the alcohol and then saturated with sodium sulphate, allowed to stand several hours and then filtered. The filtrate was again saturated with sodium sulphate and the process repeated. A black, sticky, intensely bitter mass was removed by the sulphate. The precipitate was washed with sodium sulphate solution and to the combined filtrates were added $1^1/_2$ to 2 volumes of alcohol to precipitate the sulphate. The solution was filtered and concentrated under vacuum to the original volume.

When this fluidextract was assayed by the color test it was found to be only 3% strength, but when administered to three persons it was found to be about as active as the original fluidextract.

The precipitate from the sodium sulphate was dissolved in diluted alcohol and brought up to the volume of the original fluidextract. The Borntraeger test indicated a solution 181%. It had no therapeutic activity.

This specially prepared extract had very little bitter taste, and was very much lighter in color than the original fluidextract.

SUMMARY.

The Borntraeger color reaction does not in all cases run parallel with the therapeutic activity, and is not a reliable test for the presence or activity of cascara sagrada.

A more palatable preparation may also be prepared by first extracting the cascara with alcohol, acetone, etc. But in this case there is some loss of activity. A still more palatable and active preparation can be obtained by salting out of the bitter principle by sodium sulphate. The bitterness may be thus removed without markedly impairing the therapeutic activity of the cascara.

The color-producing principle is found almost entirely in the bitter resinous substance.

Further work is being done with other salts to determine their effect on fluidextract of cascara.

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THE SEED OF EUPHORBIA MARGINATA PURSH.*

BY LOYD E. HARRIS AND MARGARET C. GALLAGHER.[1]

An investigation of the literature on plants of the Euphorbiaceæ revealed the fact that the seeds of this family yield a comparatively large amount of a fixed oil (1) which has a decided medicinal value (2). Since *Euphorbia marginata* Pursh. grows in such abundance in this section of the country a determination of the quantity and properties of the oil yielded by this species appeared to be of exceptional value and it was with this purpose in view that the present investigation was undertaken.

It was the medicinal use of these plants which occasioned the name of the family, it being named after King Juba's physician, Euphorbium, who had cured Augustus Ceasar with the gum (3). Its therapeutic use at the time was in dropsy and distemper. Since then it has been applied in a number of different diseases (4), but its use has become obsolete. The oil of several species, however, has remained as a purgative.

Of the many species of Euphorbia which have been examined, *Euphorbia lathyris* has perhaps received the greatest attention. Various analyses (5) of this plant have yielded amounts of fixed oil varying from 35 per cent to 42 per cent. This oil resembles croton oil in physiological action (6). Some other species which have been examined are *E. helioscopia* (7), *E. pillulifera* (8), *E. drummondi* (9), *E. Cyparissias* (10), *E. platyphylla* (11) and *E. resinifera* (12). The oil content of these is sometimes as low as 14 per cent and the maximum amount is rarely above 40 per cent. They nearly all yield products which act powerfully as emetics and cathartics, and in overdoses occasion dangerous if not fatal pros-

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