SCIENTIFIC SECTION

SOME OBSERVATIONS ON THE QUANTITATIVE DETERMINATION OF THE ANTHRAQUINONE DERIVATIVES IN CATHARTIC DRUGS,[1] II.

BY MUPPANNA C. TUMMINKATTI[2] WITH GEORGE D. BEAL.

INTRODUCTION.

In a previous paper[3] from this laboratory, the authors have outlined the problems involved in the accurate quantitative determination of the anthraquinone derivatives to which are ascribed, at least in part, the aperient properties of a number of widely used cathartic drugs. Much of the previously published work on this subject has had to do with the evaluation of rhubarb. Because of the very great importance of cascara in the American materia medica the authors have confined themselves to a study of this drug. The methods which have received attention in the past divide themselves into three classes: gravimetric extraction methods, gravimetric precipitation methods and colorimetric methods. Gravimetric extraction methods have been studied by Aweng,[4] Daels,[5] Tunmann,[6] Fuller[7] and Hebeisen.[8] Gravimetric precipitation methods have been investigated by Tschirch and Edner[9] and Tschirch and Pool.[10] Colorimetric methods studied have included as well methods of spectroscopic study by means of the characteristic absorption bands of the derivatives. Papers on suggested colorimetric methods of assay have appeared from the pens of Tschirch[11] and Heipe, T. and Christofoletti,[12] Bromberger,[13] Warin,[14] Maurin[15] and Fuller.[16]

After examining in detail the various types of methods proposed, the authors have taken as a basis for their work the gravimetric extraction method of Daels (*loc. cit.*) which has also been accepted as the most suitable general method by Fuller and his co-workers (*loc. cit.*). An attempt has been made to locate the sources of error in this method and one by one to introduce modifications which will have the effect of eliminating these errors, and then to weed out unnecessary steps without losing in accuracy.

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[1] Contribution from the Laboratory of Analytical Chemistry of the University of Illinois.

[2] This paper is an abstract of a portion of a thesis presented by Muppanna C. Tumminkatti in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate School of the University of Illinois.

[3] Beal and Katti, JOUR. A. PH. A., 14, 865 (1925).

[4] Aweng, *Apoth. Ztg.*, 257, 538, 1901; *Pharm. J.*, 67, 244 (1901).

[5] Daels, *Bull. acad. roy. méd. Belg.* (4) 29, 779 (1919); 30, 129 (1920).

[6] Tunmann, *Apoth. Ztg.*, 30, 493 (1915).

[7] Fuller, *J. Assoc. Official Agr. Chem.*, 5, 575 (1921); 7, 7 (1923); 8, 536 (1924).

[8] Hebeisen, "Dissertation," Bern, 135 (1916).

[9] Tschirch and Edner, *Arch. Pharm.*, 245, 150 (1907).

[10] Tschirch and Pool, *ibid.*, 246, 315 (1908).

[11] Tschirch and Heipe, *Arch. Pharm.*, 238, 427 (1900).

[12] Tschirch and Christofoletti, *Pharm. J.*, 75, 248 (1905).

[13] Bromberger, "Dissertation," Bern, 42 (1911).

[14] Warin, *J. pharm. chim.*, 21, 253 (1905); 22, 12 (1905).

[15] Maurin, *Bull. sci. pharmacol.*, 28, 373 (1921).

[16] Fuller, *loc. cit.*

It is now generally accepted that the anthraquinone derivatives in this class of drugs are only partly in the free state, and are to a large extent combined with other compounds in a fashion resembling at least the glucosidal structure. Particular evidence has been obtained along this line by the action of selective solvents and precipitating reagents. Hence in all-present day methods attempts are made to determine the anthraquinone derivatives in "free" and "combined" form. The "free" anthraquinones are those which may be extracted directly from the drug by ether, chloroform or carbon tetrachloride, the "combined" derivatives those which are only removed by these solvents after acid hydrolysis of the drug or an extract thereof.

While following the various modifications of Daels' method described in our previous paper it has been observed that the chloroform solution of the crude "free" anthraquinones contains, in addition to anthraquinone derivatives, a large amount of material which gives a gelatinous precipitate in sodium hydroxide solution, together with traces of other compounds. Furthermore, the chloroform solution of the crude "combined" anthraquinones contains material forming a gelatinous precipitate in sodium hydroxide solution, a brown substance insoluble in cold chloroform and water and a yellow waxy material soluble both in water and chloroform. Some of the properties of these substances have been observed, as follows:

Fatty Material.—This is responsible for the formation of the gelatinous precipitate which appears in the sodium hydroxide solution during the extraction of the "free" anthraquinones. It has been found to be a mixture of free fatty acids, apparently in large part arachidic acid, with a waxy material which on hydrolysis yields rhamnol and fatty acids. In this connection it may be stated that Dohme and Engelhardt[1] claim to have isolated dodecyl palmitate and stearate from cascara bark while Jowett[2] stated that the waxy material of the bark was chiefly arachidic acid and rhamnol arachidate. This finding by Jowett has been confirmed by Beal and Okey.[3] The gelatinous precipitate obtained during the purification of the "combined" anthraquinones is due chiefly to waxy material and free organic acids.

Brown Material.—This is practically insoluble in cold chloroform but slightly soluble in the hot solvent. It is practically insoluble in water, very slightly soluble in 95% alcohol but apparently more soluble in 50% alcohol. It is readily soluble in alkalies with the exception of sodium bicarbonate, the solutions being dark brown if concentrated and yellow if dilute. When the chloroform solution of the crude combined anthraquinones is shaken with sodium bicarbonate a precipitate is formed. The compound has a slight reducing action on Fehling's solution and is almost completely precipitated from its solution in dilute alcohol by lead acetate and bromine water. The compound is amorphous and swells on heating, forming a black mass which on further heating completely volatilizes. The decomposition point is between 187° and 192°. Lead acetate seems to be of some promise as a clarifier for the removal of this material. The substance is precipitated from its solution in sodium carbonate by Tschirch and Edner's diazo reagent (*loc. cit.*). The brown solution in sodium hydroxide, examined in the spectrophotometer,

[1] Dohme and Engelhardt, PROC. A. PH. A., 45, 193 (1897).

[2] Jowett, PROC. A. PH. A., 52, 288 (1904).

[3] Beal and Okey, *J. Am. Chem. Soc.*, 41, 693 (1919).

shows an absorption which is entirely different from that of any known natural or synthetic hydroxymethylanthraquinone.

No substance of this type is found in the chloroform solution during the determination of the "free" anthraquinones, hence we have concluded that this material does not itself occur in the drug but is a product of hydrolysis, the parent substance accompanying the anthraquinone either in combination therewith or as a second conjugated material. Furthermore, the drug which has been exhausted by hot water percolation in the preparation of the fluidextract does not afterwards yield any such material upon hydrolysis, leading to the further conclusion that the substance is water soluble. This parent substance is also apparently soluble in ethyl acetate.

Yellow Waxy Material.—This is soluble in water, ether and chloroform. It may be extracted by chloroform or ether from its water solution but the reverse is not true. Its solution in all alkalies is yellow to orange, and the solution in sodium hydroxide has an absorption curve which is entirely different from that of a similar solution of any known natural or synthetic hydroxymethylanthraquinone. This material, like the *brown material*, is precipitated by Tschirch's diazo reagent.

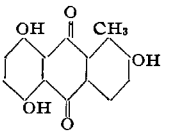
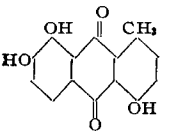
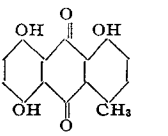
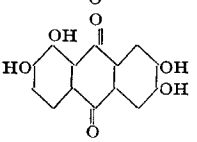
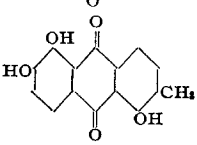
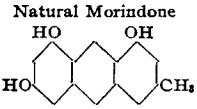
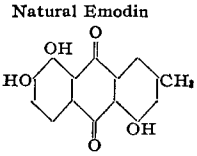
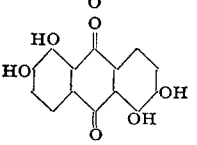
EXPERIMENTAL.

To determine the effect of the suggested clarifying agents upon the anthraquinone derivatives, a number of similar natural and synthetic compounds, the latter prepared under the direction of Professor Roger Adams, were dissolved in chloroform or ether and subjected to the following treatments: (*a*) shaking with Norit, (*b*) shaking with sodium hydroxide solution, (*c*) shaking with sodium bicarbonate solution, (*d*) shaking with ferric chloride solution, (*e*) shaking with lead acetate solution. The behavior of the various compounds with the reagents mentioned will be found in Table I.

In view of the fact that the identity of the anthraquinone derivatives present in cascara, aside from the emodin, has not been definitely established, and that the above tests have established the fact that derivatives of this nucleus may be precipitated by lead acetate, it is not yet to be assumed that this reagent is a safe clarifying medium. Accordingly the precipitate obtained by treating the chloroform solution of the impure "combined" anthraquinones with lead acetate, after washing with chloroform until free from all soluble matter, was decomposed with hydrochloric acid and the resulting solution shaken with chloroform. The chloroform extract was dark red and gave a reddish pink color with sodium hydroxide, indicating the possible loss of hydroxymethylanthraquinones during the clarification with lead acetate.

It also appeared from the above tabulation that the use of sodium bicarbonate as a clarifier was attended with some danger of loss. Fuller, however, in his latest communication, *loc. cit.*, proposes this as a standard treatment. The chloroform extract of the hydrolyzed drug after shaking with the bicarbonate solution was filtered, the solvent distilled and the residue dissolved in sodium hydroxide solution. This solution was mixed with filter pulp and the mixture filtered and washed. The alkaline filtrate was then acidified, allowed to stand and filtered, and the precipitate after drying extracted with chloroform in a Soxhlet extractor. The residue thus obtained contained only a trace of the *brown material.*

TABLE I.—ACTION OF CLARIFYING AGENTS UPON NATURAL AND SYNTHETIC ANTHRAQUINONES.

Compound.	Color of solution.	Action of Norit.	Color of alkaline sol.	Action of $NaHCO_3$.	Action of $FeCl_3$.	Action of $Pb(C_2H_3O_2)_2$.
	Yellow[1]	Decolorized	Magenta	No ppt. or color	No ppt. or color	No ppt.
	Yellow[1]	Decolorized	Violet	No ppt. or color	No ppt. or color	Dirty brown to pink ppt.
	Orange[1]	Decolorized	Blue-violet	No ppt. or color	No ppt. or color	No ppt.
	Yellow[2]	Decolorized	Purple	No ppt. Aqueous sol. red	No ppt. or color	Rose ppt. in aqueous layer, ether colorless
 Natural Morindone	Yellow[1]	Decolorized	Purplish violet	No ppt. or color	No ppt. or color	Dark purple ppt.
 Natural Emodin	Yellow[1]	Decolorized	Purplish pink	No ppt. or color	No ppt. or color	No ppt. or color
	Yellow[1]	Decolorized	Purplish violet	No ppt. or color	No ppt. or color	Violet ppt. ether decolorized
 Rufiopin	Yellow[2]	Decolorized	Deep blue	Blue-violet no ppt.	No ppt. or color	Blue-violet ppt.

[1] Chloroform solution. [2] Ether solution.

Mention was made in our previous paper and also in the above table of the complete adsorption of the anthraquinones by shaking their solution with Norit. Accordingly, chloroform extracts of both the "free" and "combined" anthraquinones were shaken with Norit and filtered, and the residues weighed after distillation of the solvent. These residues, each representing five grams of drug, were, after weighing, dissolved in neutral 95% alcohol, and titrated with alkali having a normality of 0.0941, using phenol-phthalein as the indicator.

Analysis. Residues from "free" anthraquinones, 0.0942 Gm., 0.0929 Gm.; cc. alkali, 1.62, 1.59. Residues from "combined" anthraquinones, 0.0212 Gm., 0.0213 Gm.: cc. alkali, 0.48, 0.50.

This residue evidently contains a fatty acid or similar substance and as such will accompany the anthraquinones through all of the stages of chemical clarification. The final adsorption of the anthraquinones by Norit has been adopted as a standard procedure in our assay method.

Daels, *loc. cit.*, made use of kieselguhr as a means of mechanically removing some of the brown impurity. To determine the possibility of the loss of any anthraquinones, samples of emodin were dissolved in chloroform and the solution shaken with kieselguhr, then filtered and an aliquot part of the filtrate evaporated for the determination of the unabsorbed emodin.

TABLE II.—ADSORPTION OF EMODIN BY KIESELGUHR.

Weight emodin taken.	Weight emodin recovered.	Loss of emodin.
0.0200	0.0190	5.0%
0.0200	0.0189	5.5%
0.0200	0.0189	5 5%

This loss of emodin, it is true, may be balanced to some extent by the presence of some of the *brown material* not removed by the kieselguhr.

In our previous paper we have outlined a number of modifications of the Daels method which were used in the analysis of cascara bark with varying degrees of success. The following modifications follow in sequence those of the previous paper, and as in the previous paper the results obtained with these modified assay processes will be given in one table for purposes of comparison. The reader will bear in mind that in all of these determinations the same lot of cascara bark has been used.

Chemical Clarification. *Procedure G.*—This was essentially Daels' method with slight modification.

A five-gram sample of drug was refluxed with 250 cc. of chloroform for thirty minutes, cooled, filtered and washed with chloroform. The extract was alternately shaken with 20-cc. portions of 5% sodium hydroxide and water until no further color was removed. The alkaline extracts were filtered through hardened filter paper, acidified with hydrochloric acid and shaken out with repeated small portions of chloroform. The chloroform was distilled from a tared vessel and the residue weighed as impure "free" anthraquinones. This residue was then dissolved in 100 cc. of chloroform, shaken with one gram of Norit, and after filtering and washing the Norit the solvent was distilled from the same tared vessel. The residue was again dried for one hour at 100° and weighed. The weight of the impurities was subtracted from that of the impure residue, the difference being taken as the weight of pure "free" anthraquinone.

The residual drug was now refluxed with 250 cc. of chloroform and 50 cc. of 25% sulphuric acid for two and one-half hours. After replacing the chloroform lost by evaporation 200 cc. of the extract was filtered off and alternately shaken with 20-cc. portions of 5% sodium hydroxide and water until exhausted. The combined extracts were filtered through hardened paper, acidified with hydrochloric acid and then extracted with repeated small portions of chloroform. The chloroform extract was shaken with 100 cc. of 5% sodium bicarbonate solution, which after separation was twice washed with chloroform and the washings added to the chloroform solution. The chloroform solution was then evaporated in a tared dish, dried as above, and weighed as crude "combined" anthraquinones. The weight of pure anthraquinones was then determined by the above Norit treatment.

In the extraction of the "free" anthraquinones from the acidified aqueous solution the chloroform left a slight insoluble residue in the aqueous solution. It was also noticed that in the chloroform extraction of the "combined" anthraquinones

just before the bicarbonate treatment some of the *brown material* was left in the water solution although the last chloroform extract was colorless. The aqueous solution also retained a yellow color, but upon the addition of alkali only a slight brown tint developed.

The chloroform extract before the bicarbonate treatment was bright red, although the original chloroform solution was orange-yellow. The solution became yellow again upon shaking with the bicarbonate, while the bicarbonate became brownish red and some of the *brown material* separated, leaving the bicarbonate solution cloudy, making it appear that this material was in suspension rather than in solution.

Procedure H.—This was identical with *G* except that the chloroform solution was diluted to 200 cc. before the bicarbonate treatment and afterwards an aliquot of 150 cc. was taken to complete the assay.

Procedure I.—In this Daels' method was followed exactly, adding the Norit treatment for the purification of the "free" and "combined" anthraquinones. It was noted that the fatty residue was greater in quantity than in almost all of the other procedures, and also that the Norit adsorbed any of the *brown* and *yellow waxy materials* not otherwise removed, causing them to be determined as anthraquinones.

Procedure J.—Thinking that the *brown material* might be a product of hydrolysis of the tannins, the chloroform solution after the acid hydrolysis for the extraction of "combined" anthraquinones was shaken with 100 cc. of 5% ferric chloride solution and the final chloroform solution shaken with 50 cc. of 5% ferric chloride instead of 100 cc. of 5% sodium bicarbonate. The ferric chloride solution from the first treatment was dark brown and contained a black precipitate.

In order to determine whether this treatment removed anthraquinones the ferric chloride was shaken with chloroform, which was rejected, then made distinctly acid with hydrochloric acid. Shaking this solution with chloroform failed to remove any anthraquinone, as shown by the test with sodium hydroxide. However, if the solution after acidifying was warmed on the steam-bath for thirty minutes and then extracted with chloroform, some anthraquinone was found in the chloroform. Evidently the ferric chloride removed some of the anthraquinone as a fairly stable compound and was a potential source of error. The brown material, however, was almost completely removed.

Mechanical Purifications. *Procedure K.*—(*a*) Attempts were made to extract the "combined" anthraquinones by means of ether, benzene and chloroform containing dry hydrochloric acid. More of the undesirable material was extracted than in the sulphuric acid-chloroform procedure. (*b*) Both ether and chloroform were used as solvents to remove the "free" anthraquinones in a Soxhlet extractor. The drug in the thimble was then moistened with 5 cc. of concentrated hydrochloric acid or 25% sulphuric acid and again extracted. After this extraction the drug was subjected to the usual sulphuric acid-chloroform treatment but no more anthraquinone was obtained. However, more of the foreign material was extracted.

(*c*) The "free" anthraquinones were extracted as usual and the "combined" anthraquinones extracted by chloroform during the acid hydrolysis. After distilling the chloroform the residue was dissolved in 5% sodium hydroxide, acidified with hydrochloric acid and the precipitate filtered after settling. The precipitate was washed free from acid, dried and extracted with chloroform in a Soxhlet extractor. The extract was dark red and the thimble contained an appreciable quantity of the *brown material*. By a repetition of the procedure with this chloroform extract very little *brown material* was left on the paper.

(*d*) Essentially the same procedure was followed except that the first chloroform solution was shaken with sodium hydroxide instead of the evaporation and solution previously used. Practically all of the *brown material* was removed in this way.

Procedure L.—This method was tried in a quantitative way. "Free" anthraquinones were determined as usual. The chloroform solution of the "combined" anthraquinones was extracted

with sodium hydroxide with intermediate washings, the extract filtered, acidified and shaken out with chloroform. The crude "combined" anthraquinones were determined by evaporation and weighing, then purified by the Norit treatment.

Procedure M.—The final chloroform solution of "combined" anthraquinones obtained as in *L* was again extracted with sodium hydroxide and treated as above. The final chloroform extract in *L* was distinctly red and the anthraquinone brown. The aqueous solution after the extraction of the anthraquinone retained a considerable quantity of insoluble brown material. The double purification as in *M* gave much better results. However, the anthraquinones still retained some of the *yellow waxy material.*

Procedure N.—"Free" anthraquinones were determined by the usual method, using five grams of drug. The drug was then hydrolyzed with 50 cc. of 25% sulphuric acid in the presence of 250 cc. of chloroform, and an aliquot of 200 cc. filtered and distilled. The residue was dissolved in 20 cc. of 5% sodium hydroxide and diluted to 200 cc. with water, acidified with hydrochloric acid, allowed to settle for thirty minutes and then filtered through hardened filter paper. The precipitate was dissolved back into the original vessel by alternate treatments with 5%

TABLE III.—"FREE" AND "COMBINED" ANTHRAQUINONES AS DETERMINED BY VARIOUS MODIFICATIONS OF DAELS' METHOD.

Method.	Wt. impure "free" anthraquinones.	Wt. impurity.	Wt. pure "free" anthraquinones.	% Free anthraquinones.	Wt. impure "combined" anthraquinones.	Wt. impurity.	Wt. pure "combined" anthraquinones.	% "Combined" anthraquinones.	
G1*	0.0101	0.0040	0.0061	0.12	0.0447	0.0032	0.0415	1.03	
G2*	0.0078	0.0031	0.0047	0.09	0.0425	0.0028	0.0397	0.99	
G3	0.0167	0.0048	0.0119	0.24	....	....	....	..	
G4	0.0252	0.0091	0.0161	0.32	0.0371	0.0026	0.0345	0.86	
H1	0.0080	0.0028	0.0052	0.10	0.0273*	0.0043	0.0230	0.77	
H2	0.0078	0.0028	0.0050	0.10	0.0270*	0.0052	0.0218	0.73	
I1	0.0053	0.0005	0.0048	0.19	0.0381	0.0058	0.0323	2.15	2.54[1]
I2	0.0053	0.0007	0.0046	0.18	0.0371	0.0066	0.0305	2.03	2.47
I3	....	....	....	..	0.0679	0.0092	0.0587	1.96[2]	2.26[2]
I4	....	....	....	..	0.0710	0.0077	0.0633	2.11[2]	2.37[2]
I5	0.0041	0.0012	0.0029	0.12	0.0438	0.0085	0.0353	2.35	2.92
I6	0.0034	0.0004	0.0030	0.12	0.0447	0.0126	0.0321	2.14	2.98
I7	0.0052	0.0014	0.0038	0.15	0.0416	0.0099	0.0317	2.11	2.77
I8	0.0078	0.0019	0.0059	0.23	0.0421	0.0092	0.0329	2.19	2.81
I9	0.0084	0.0018	0.0066	0.26	0.0442	0.0086	0.0356	2.37	2.95
I10	0.0081	0.0021	0.0060	0.24	0.0447	0.0107	0.0340	2.26	2.98
J1	0.0084	0.0029	0.0055	0.11	0.0373	0.0048	0.0325	1.08	
J2	0.0259	0.0144	0.0113	0.22	0.0356	0.0055	0.0301	1.00	
J3	0.0170	0.0064	0.0106	0.21	0.0375	0.0066	0.0309	1.03	
J4	0.0172	0.0062	0.0110	0.22	0.0373	0.0063	0.0310	1.03	
J5	0.0134	0.0046	0.0088	0.18	0.0357	0.0053	0.0304	1.01	
J6	0.0131	0.0035	0.0096	0.19	0.0355	0.0048	0.0307	1.02	
J7	0.0161	0.0066	0.0095	0.19	0.0341	0.0048	0.0293	0.98	
J8	0.0166	0.0064	0.0102	0.20	0.0350	0.0052	0.0298	0.99	
L1	0.0230	0.0101	0.0129	0.26	0.0806	0.0084	0.0722	1.80	
L2	0.0238	0.0120	0.0118	0.23	0.0748	0.0064	0.0684	1.71	
L3	0.0224	0.0092	0.0132	0.26	0.0860	0.0154	0.0706	1.76	
L4	0.0255	0.0127	0.0128	0.25	0.0770	0.0081	0.0689	1.72	
M1	0.0181	0.0052	0.0129	0.26	0.0608	0.0073	0.0535	1.34	
M2	0.0176	0.0057	0.0117	0.23	0.0633	0.0080	0.0553	1.38	
M3	0.0209	0.0088	0.0121	0.24	0.0634	0.0092	0.0544	1.36	
M4	0.0203	0.0101	0.0102	0.20	0.0631	0.0089	0.0542	1.35	
M5	0.0222	0.0096	0.0126	0.25	0.0675	0.0108	0.0567	1.42	
M6	0.0209	0.0096	0.0113	0.23	0.0664	0.0100	0.0564	1.41	
M7	0.0220	0.0089	0.0131	0.26	0.0592	0.0081	0.0511	1.28	
M8	0.0216	0.0105	0.0111	0.22	0.0576	0.0079	0.0497	1.24	
N1	0.0182	0.0081	0.0101	0.20	0.0391	0.0034	0.0357	0.89	
N2	0.0162	0.0067	0.0095	0.19	0.0396	0.0027	0.0369	0.92	
N3	0.0177	0.0063	0.0114	0.23	0.0402	0.0036	0.0366	0.91	
N4	0.0152	0.0056	0.0096	0.19	0.0393	0.0031	0.0362	0.90	

* The chloroform extracts were not washed with water during the extraction with sodium hydroxide.

[1] Values in this column are those obtained by Daels' procedure unmodified.

[2] The solution of the entire 5-gram sample was used.

sodium hydroxide and water, again acidified and extracted with chloroform. The chloroform solution was extracted with sodium hydroxide, alternating by washing with water, and the extract filtered, acidified with hydrochloric acid and shaken out with chloroform, the chloroform extract being washed with a small amount of water. The solvent was distilled from a tared vessel and the residue weighed, after drying at 100° for one hour, as crude "combined" anthraquinones. The usual Norit treatment was then used to obtain the weight of pure residue.

The *yellow waxy material* was found in the first acid filtrate. This could be extracted by chloroform, which was done in two instances, the solvent distilled and the residue weighed. The two samples yielded respectively 0.0222 Gm. and 0.0220 Gm., which corresponded to 0.55% of the drug. This residue dissolved in sodium hydroxide to a yellow or orange solution, which after standing for ten hours developed a faint pink tint, although the yellow still predominated. It is possible that this contains a small amount of an anthranol derivative which is gradually oxidized to anthraquinone on standing in the air.

After dissolving the precipitated anthraquinones from the filter paper with alkali some brown fatty material was left on the filter. Most of the *brown material* was left in suspension in the acid aqueous solution after the next chloroform extraction. After the final chloroform extraction the last of the *brown material* was left in the aqueous layer.

The combined anthraquinones turned quite brown on drying but dissolved in sodium hydroxide to give a pink solution with only the merest trace of yellow tint. The color of this alkaline solution when viewed with the unaided eye appeared to match exactly that obtained by the solution of pure emodin in alkali. When observed through the colorimeter, however, a distinct difference in tint could be seen.

THE ABSORPTION SPECTRA OF THE ANTHRAQUINONE DERIVATIVES.

With the kind assistance of Mr. I. H. Godlove the spectra of the various residues and solutions were examined in a Keuffel and Esser Spectrophotometer. It was hoped by this means to determine the degree of purity of the residues at the various stages of the analysis, since entirely different absorption curves would be given by anthraquinones and non-anthraquinone material. The measurements shown are quantitative for the transmission of the various wave-lengths of light by any one solution but do not accurately represent concentrations of solid in the solution. For this reason they are only available as an index of the degree of purification and cannot serve to measure the amount of anthraquinone in the drug.

In the plotting of the spectra of these solutions which has been done on logarithmic cross-section paper the following terms are used.[1]

The *transmission*, T, of the cell and contents is defined as the ratio of the radiant power passing the last surface of the cell to that incident on the first surface.

The *transmittency*, T^1, of the contents of the cell is the ratio of the radiant power incident on the second inner surface of the cell to that passing the first inner surface. (Absorption = $1-T^1$.)

Preparation of the Solutions.—The solutions used are intended to represent the different stages of the extraction and purification of the anthraquinone derivatives. For comparative purposes the extractions were repeated with a specimen of emodin of known purity. Solutions of the various impurities were also prepared for examination. The concentration of each solution as obtained was arbitrarily taken as 1.

Five grams of drug was refluxed with 250 cc. of chloroform for thirty minutes. The mixture was cooled and filtered and the residue washed with enough chloroform

[1] Scientific Paper No. 440, U. S. Bureau of Standards.

to make the filtrate measure 250 cc. 100 cc. of this solution was reserved for examination. Curve I.

The remaining 150 cc. of chloroform extract was shaken out with alkali with intermediate washings, the extract filtered, acidified with hydrochloric acid and shaken out with chloroform. The chloroform extract was filtered and made up to 150 cc., 100 cc. being reserved for examination. Curve II.

The residual drug was hydrolyzed with 250 cc. of chloroform and 50 cc. of 25% sulphuric acid for three hours, then cooled and the lost chloroform restored. Two hundred cc. of the chloroform extract was filtered and 100 cc. reserved for examination. Curve III.

The remaining 100 cc. was extracted with alkali as usual and the alkaline extract filtered, acidified with hydrochloric acid and extracted with chloroform. The chloroform solution was again extracted with alkali and the extract filtered, then acidified and extracted with chloroform. This chloroform extract was concentrated to 100 cc. for Curve IV.

Fifteen-hundredths gram of pure emodin was dissolved in 300 cc. of warm chloroform, the solution cooled and made up to 500 cc. 100 cc. of this solution gave Curve V.

Two hundred and fifty cc. of the remaining solution was refluxed with 50 cc. of 25% sulphuric acid for three hours, then cooled and the lost chloroform restored. Two hundred cc. of the solution was filtered and 100 cc. taken for Curve VI.

The remaining 100 cc. was extracted with alkali, acidified and extracted with chloroform. The chloroform extract was filtered and evaporated to 100 cc. then used for Curve VII.

A second series of solutions was prepared exactly as described above, but in each case the final chloroform solution was evaporated, the residue dissolved in 20 cc. of 5% sodium hydroxide and made up to 100 cc. with water. The curves given by these alkaline solutions accompany those from the chloroform solutions.

Four additional alkaline solutions were prepared as follows:

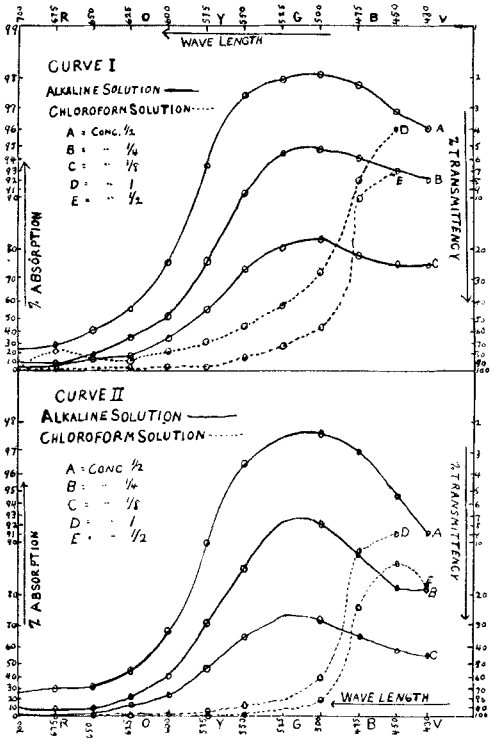
A portion of the pure *brown material* weighing 0.03 Gm., freed from anthraquinones by extraction with chloroform and ether, was dissolved in 20 cc. of 5% sodium hydroxide and diluted to 100 cc. with water. This gave Curve VIII.

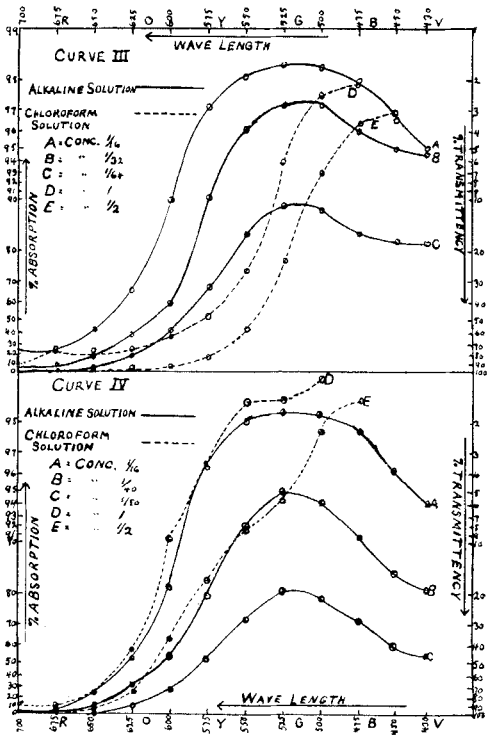
A mixture of 0.015 Gm. each of emodin and *brown material* was dissolved in 20 cc. of 5% sodium hydroxide and diluted to 100 cc. with water. The data is shown in Curve IX.

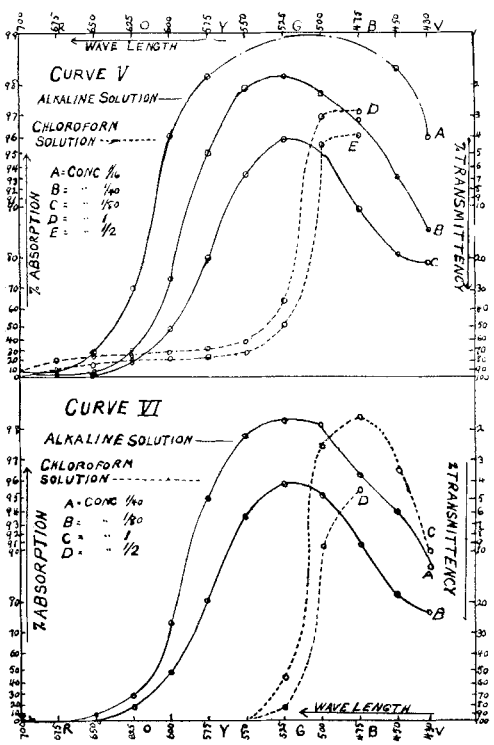
According to procedure *N*, the final chloroform extract of the "combined" anthraquinones was obtained. This was diluted to 200 cc., representing 4 Gm. of drug, and 100 cc. of the solution evaporated to dryness. The residue was dissolved in 20 cc. of 5% sodium hydroxide and diluted to 100 cc. with water. The data are found in Curve X.

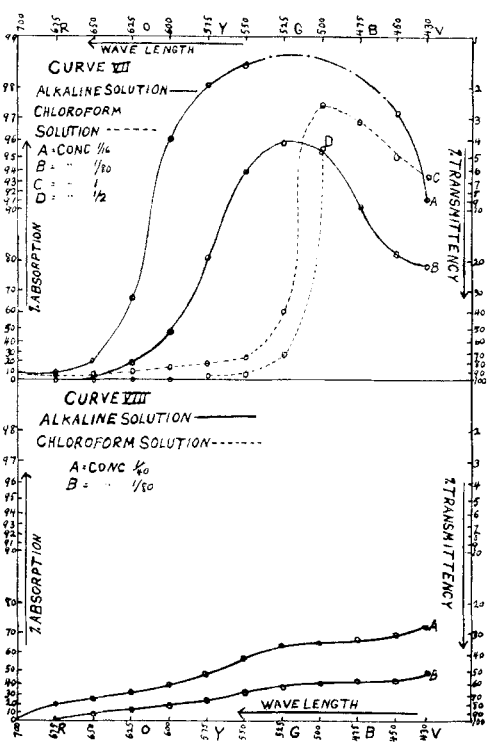
The aqueous acidic filtrate from the above sample containing the *yellow waxy material* was extracted with chloroform and the chloroform solution evaporated to dryness. The residue, representing 4 Gm. of drug, was dissolved in 20 cc. of 5% sodium hydroxide, the solution diluted to 100 cc. with water and examined, the data furnishing Curve XI.

Discussion of Curves.—Little attention can be paid to the absorption curves obtained in chloroform solution because of their irregularity. Of those obtained


CURVE I
ALKALINE SOLUTION
CHLOROFORM SOLUTION
A = Conc. 1/2
B = " 1/4
C = " 1/8
D = " 1
E = " 1/2
% ABSORPTION
% TRANSMITTENCY
WAVE LENGTH
CURVE II
ALKALINE SOLUTION
CHLOROFORM SOLUTION
A = Conc 1/2
B = " 1/4
C = " 1/8
D = " 1
E = " 1/2
% ABSORPTION
% TRANSMITTENCY
WAVE LENGTH


CURVE III
ALKALINE SOLUTION
CHLOROFORM SOLUTION
A = Conc. 1/4
B = " 1/32
C = " 1/64
D = " 1
E = " 1/2
% ABSORPTION
% TRANSMITTENCY
WAVE LENGTH
CURVE IV
ALKALINE SOLUTION
CHLOROFORM SOLUTION
A = Conc. 1/4
B = " 1/40
C = " 1/80
D = " 1
E = " 1/2
% ABSORPTION
% TRANSMITTENCY
WAVE LENGTH


CURVE V
ALKALINE SOLUTION
CHLOROFORM SOLUTION
A = Conc 1/4
B = " 1/40
C = " 1/80
D = " 1
E = " 1/2
% ABSORPTION
% TRANSMITTENCY
WAVE LENGTH
CURVE VI
ALKALINE SOLUTION
CHLOROFORM SOLUTION
A = Conc 1/40
B = " 1/80
C = " 1
D = " 1/2
% ABSORPTION
% TRANSMITTENCY
WAVE LENGTH


CURVE VII
ALKALINE SOLUTION
CHLOROFORM SOLUTION
A = Conc 1/4
B = " 1/80
C = " 1
D = " 1/2
% ABSORPTION
% TRANSMITTENCY
WAVE LENGTH
CURVE VIII
ALKALINE SOLUTION
CHLOROFORM SOLUTION
A = Conc 1/40
B = " 1/80
% ABSORPTION
% TRANSMITTENCY
WAVE LENGTH

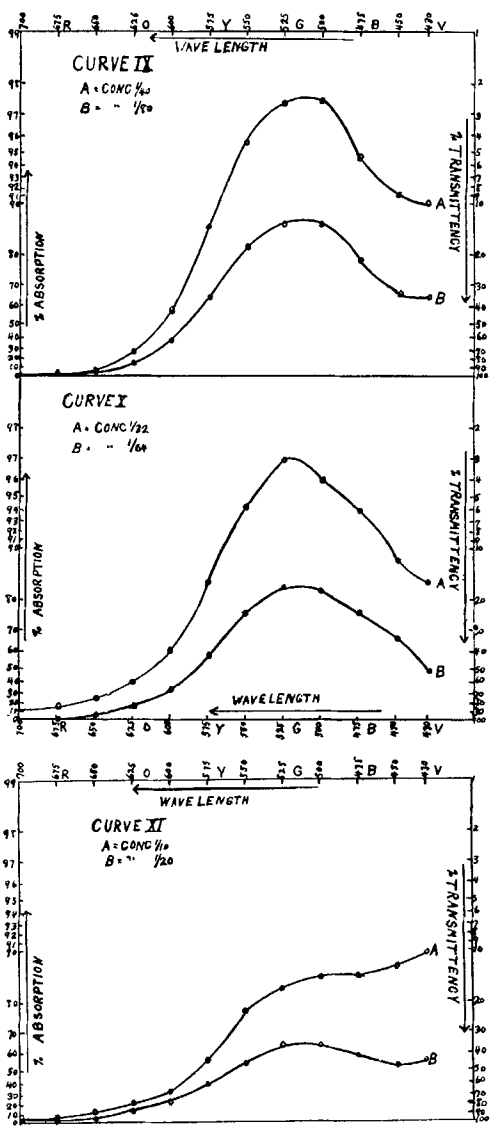
with the sodium hydroxide solutions, the curves I to VII and IX and X show maximum absorption at the green line, indicating that the color to the eye will be purple.

Curve V, which is characteristic of emodin and most of the other hydroxymethylanthraquinones, shows a sharp maximum at the green line, indicating a purple or pink solution. Curves VI and VII follow V very closely, suggesting that emodin is not affected in any way during the analytical procedure. This has been verified by gravimetric and colorimetric determinations.

In Curves I and II will be noticed a difference in absorption in the region of the blue and violet lines. Curve I shows a gradual change in absorption, yielding practically a straight line, while Curve II shows a pronounced slope. This indicates that the color of the solution represented by Curve I will have more yellow than that represented by Curve II. This is an indication of the partial purification.

In Curves III and IV the removal of the *yellow waxy material* becomes apparent. The *brown material* in low concentrations shows practically no absorption, hence does not affect the curves when present.

Curve IX, representing an equal mixture of emodin and *brown material*, shows that the solution, as far as color is concerned, is of practically pure emodin. It might be noted that concentrations 1/80 in Curve V and 1/40 in Curve IX show practically identical absorption and transmission for the various lines. These solutions as examined have the same concentration of emodin.



In comparing Curves IV and X it will be found that X has a sharp maximum absorption at the green line while IV has a gradual slope, showing more of a purple tint. Curves VIII and XI definitely indicate that these substances are not hydroxymethylanthraquinones.

RECOMMENDED METHOD FOR ASSAY.

Preparation of the Sample.—The drug for assay should be in the form of powder, not coarser than No. 40. Liquid preparations should be pipetted onto shredded filter paper or purified saw-

dust in a porcelain dish and carefully dried on the steam-bath. After transferring the dried material to the extraction flask the dish should be wiped out with small pieces of moistened filter paper which are subsequently dried and placed in the flask

Determination of "Free" Anthraquinones.—Place five grams of drug, or 5 cc. of liquid preparation prepared as above in a 500-cc. Erlenmeyer flask, add 250 cc. of chloroform and weigh the flask and contents. Connect the flask with a reflux condenser and heat for one hour, keeping the chloroform boiling gently. Cool the mixture, filter and wash the residue with several small portions of chloroform. Transfer the chloroform extract to a separatory funnel and extract alternately with 20-cc. portions of 5% sodium hydroxide and water until the extracts no longer have any pink tint. Filter the alkaline extracts and washings through hardened filter paper.

Acidify the filtrate with hydrochloric acid and extract repeatedly with small portions of chloroform, washing the combined extracts once with water and then filtering into a tared vessel. Distil off the chloroform and dry the residue at 100° for one hour. Weigh as impure "free" anthraquinones.

Dissolve the weighed residue in 100 cc. of chloroform and shake the solution with one gram of Norit which has been previously extracted with chloroform for twelve hours. Filter the solution into the vessel previously used, wash the residue with several small portions of chloroform, evaporate the solvent and dry the residue at 100° for one hour. Weigh the dish and impurities and subtract from the previous weight. The difference will represent the weight of pure "free" anthraquinones.

Determination of "combined" anthraquinones. Return the residual drug, together with the filter paper, to the extraction flask, and add chloroform to the original weight. Add 50 cc. of 25% sulphuric acid and weigh again. Reflux the mixture at the boiling point of the chloroform for two and one-half hours, cool and weigh again, then restore any chloroform lost by evaporation. Filter the chloroform layer and measure out a 200-cc. portion. Evaporate the solvent in a small flask or beaker. (The residue should have an orange-yellow color.) Dissolve the residue in 20 cc. of 5% sodium hydroxide and dilute the solution to 200 cc. with distilled water. Acidify the solution with hydrochloric acid and allow to stand for thirty minutes. Filter the precipitate on a hardened paper and wash once with acidulated water. Dissolve the precipitate from the paper into the precipitation flask with alternate portions of 5% sodium hydroxide and distilled water. Acidify the aqueous solution with hydrochloric acid and extract the anthraquinones with repeated small portions of chloroform. Shake out the combined chloroform extracts with alternate portions of 5% sodium hydroxide and water, combine the extracts, acidify with hydrochloric acid and again extract with chloroform. Distil the chloroform from a weighed vessel, dry at 100° for one hour and weigh as impure "combined" anthraquinones. Determine the "pure" anthraquinones as in the previous operation.

The results for the analysis of cascara bark according to the recommended procedure will be found described as experiments N1 to N4 in Table III. In addition five preparations of cascara bark were analyzed by the same method, the results being given in Table IV. A is a specimen of Fluidextract of Cascara, U. S. P. IX, prepared by the authors. B is a commercial sample of the fluidextract prepared by a prominent manufacturer. C is a specimen of fluidextract from another manufacturer. D is a debitterized preparation from the manufacturer of C. E is a specimen of resin said to be removed during the debitterizing of D and itself used medicinally. F is a specimen of exhausted bark remaining after the manufacture of A.

Specimens A, B and C when diluted with water became turbid and a brown precipitate separated. While extracting the chloroform solution with sodium hydroxide the layers separated cleanly with no formation of emulsions. This is thought to be due to the practically complete absence of fatty material owing to the nature of the menstruum used. The major portion of the solids of the fluidextracts seemed to consist of the parent substance of the *brown material*, although

there is also present an appreciable quantity of the parent substance of the *yellow waxy material.*

Specimen D when diluted with water gave a perfect solution even after heating on the steam-bath. When the residue which was weighed as "combined" anthraquinones was dissolved in sodium hydroxide no indication of a pink color could be seen. For all practical purposes it may be considered that this preparation does not contain hydroxymethylanthraquinones. This has also been confirmed by one of the authors in a study of the qualitative methods for the identification of cathartic drugs. The major part of the solids in the preparation seems to consist of the parent substances of the *brown material* and the *yellow waxy material.*

During the analysis of Specimen E qualitative tests showed the presence of the *yellow waxy material* in the residue of "free" anthraquinones. Accordingly in this part of the procedure the alkaline solution of the impure "free" anthraquinones was acidified and after settling was filtered. The precipitate was dissolved from the filter with 5% sodium hydroxide as in the determination of "combined" anthraquinones and the determination completed. The presence of the parent substances of the *brown material* and of the *yellow waxy material* was demonstrated in considerable amounts.

The crude "combined" anthraquinones from Specimen F were observed to contain only very small amounts of the *brown material* and of the *yellow waxy material.*

TABLE IV.—ANALYSIS OF CASCARA PREPARATIONS.

Lot.	Impure "free" anthra quinones.	Impurity.	Pure "free" anthra-quinones.	% "Free" anthra-quinones.	Impure "combined" anthra-quinones.	Impurity	Pure "combined" anthra-quinones.	% "Combined" anthra-quinones.
A1	0.0044	0.0007	0.0037	0.07	0.0170	0.0008	0.0162	0.40
A2	0.0039	0.0005	0.0034	0.07	0.0181	0.0010	0.0171	0.43
A3	0.0048	0.0017	0.0031	0.06	0.0176	0.0010	0.0166	0.41
B1	0.0051	0.0015	0.0036	0.07	0.0071	0.0003	0.0068	0.17
B2	0.0043	0.0012	0.0031	0.06	0.0070	0.0004	0.0066	0.16
B3	0.0052	0.0010	0.0042	0.08	0.0070	0.0011	0.0059	0.15
B4	0.0057	0.0013	0.0044	0.09	0.0068	0.0017	0.0051	0.13
C1	0.0062	0.0025	0.0037	0.07	0.0126	0.0031	0.0095	0.24
C2	0.0050	0.0024	0.0026	0.05	0.0133	0.0029	0.0104	0.26
C3	0.0051	0.0023	0 0028	0.06	0.0128	0.0032	0.0096	0.24
D1					0.0039	0.0011	0.0028	0.07
D2	This preparation contains no free anthra-				0.0044	0.0013	0.0031	0.08
D3	quinones				0.0040	0.0015	0.0025	0.06
D4					0.0039	0.0013	0.0026	0.06
E1*	0.0041	0.0010	0.0031	0.25	0.0188	0.0014	0.0174	1.74
E2*	0.0050	0.0012	0.0038	0.30	0.0189	0.0016	0.0173	1.73
E3*	0.0047	0.0012	0.0035	0.28	0.0184	0.0017	0.0167	1.67
F1	0.0230	0.0082	0.0148	0.30	0.0101	0.0024	0.0077	0.19
F2	0.0201	0.0071	0.0130	0.26	0.0097	0.0023	0.0074	0.18

* This material, being a dry powder, was treated in exactly the same fashion as the crude drug, a sample weighing 1.25 Gm. being taken for analysis.

SUMMARY.

The gravimetric extraction method for the assay of cascara bark, based upon the work of Daels, has been adopted as the most feasible process for the determination of the hydroxymethylanthraquinones present in this important drug.

Three distinct types of substances have been recognized as contaminating the final residue of anthraquinones obtained in the original process. These are as follows:

(*a*) A fatty residue which consists of fatty acids of the nature of arachidic acid, together with phytosterol esters of this acid. The acid accompanies the hydroxyanthraquinones through all of the stages of purification because of the similar acidic properties.

(*b*) A brown substance practically insoluble in chloroform and water and apparently formed by hydrolysis during the extraction of the "combined" anthraquinones. This substance is soluble in strong alkalies and is precipitated by lead acetate and by Tschirch's diazo reagent.

(*c*) A yellow waxy substance, probably formed during hydrolysis. This substance is soluble in water, ether and chloroform, and is extracted from a water solution by ether or chloroform, but not *vice versa*. This substance is also precipitated by Tschirch's diazo reagent.

The hydroxymethylanthraquinones are quantitatively adsorbed from chloroform or ether by Norit decolorizing carbon, but the fatty impurity is untouched. This reagent may be used for purification of the final anthraquinone residue.

Reagents which precipitate the *brown* and the *yellow waxy material* were found to react in some cases with hydroxyanthraquinones, forming a potential source of error. The most satisfactory method for the separation of these substances relies only upon the acidic nature of the hydroxymethylanthraquinones.

In the extraction of the anthraquinones from their solution in chloroform by shaking with sodium hydroxide solution it is expedient to use alternate portions of reagent and water, since the number of extractions required is thereby reduced.

Examination of all of the residues in the spectrophotometer shows that the contaminating substances are probably not anthraquinone derivatives. These readings further show that it will be exceedingly difficult to obtain a satisfactory reproducible color standard for the colorimetric assay of these drugs.

Results obtained during the study of these assay methods prove beyond a doubt that the larger part of the anthraquinone derivatives in cascara bark are present in the combined form.

A simple and accurate method for the assay of cascara bark and its preparations is proposed.

URBANA, ILLINOIS.

FIFTIETH ANNIVERSARY OF JOHNS HOPKINS.

Distinguished scientists and scholars from foreign universities and many alumni from all parts of the world will attend the two-day celebration of the fiftieth anniversary of the Johns Hopkins University on October 22 and 23.

The main events of the celebration will be: Addresses on scientific subjects by representatives of English, French and German universities; exercises commemorating the founding of the university in 1876; dedication of the new $1,000,000 building of the School of Hygiene and Public Health, and conferences in sixteen branches of science and scholarly learning.

Dr. Andrew Balfour, director of the London School of Hygiene and Tropical Medicine, will make a special trip from England to deliver the main address at the dedication of the school on October 22. He will speak on "Hygiene as a World Force," emphasizing the important part being played by preventive medicine in the progress of modern civilization.

The commemoration exercises will begin at 10.30 A.M., October 22 at the Lyric Theater with an address of welcome by Governor Ritchie and introductory remarks by Dr. William H. Welch, director of the Johns Hopkins School of Hygiene and Public Health.