Saponin or sapogenin solution.		2.5% suspension of rabbit R. B. C. in .85% salt.	.85% salt solution.
Tube I	2.5 Cc.	0.5 Cc.	2.0 Cc.
Tube II	2.0 Cc.	0.5 Cc.	2.5 Cc.
Tube III	1.5 Cc.	0.5 Cc.	3.0 Cc.
Tube IV	1.0 Cc.	0.5 Cc.	3.5 Cc.
Tube V	0.5 Cc.	0.5 Cc.	4.0 Cc.
Tube VI	0.0 Cc.	0.5 Cc.	4.5 Cc.

Solution A. No hemolysis in any of the tubes.

Solution B. No hemolysis in any of the tubes.

Solution C. Total hemolysis in tubes 1, 2, 3, 4 and 5 after 45 minutes. No hemolysis in tube 6.

Solution D. No hemolysis in any of the tubes.

Solution E. Total hemolysis in tubes 1, 2, 3, 4 and 5 after 15 minutes. No hemolysis in 6.

The above experiments were carried out in duplicate, one set being upon drug obtained from *Digitalis purpurea*, the second on *Digitalis Siberica*, both from plants grown in the Stearns Medicinal Plant Gardens at the University of Michigan.

These results appear to support the view originally put forward by Kobert, that any hemolytic activity of the leaf-drug is due to sapogenins resulting from hydrolysis of the saponins. It may be possible that the saponins of improperly dried or stored drug undergo sufficient decomposition so that preparations from such drug will exhibit hemolytic action.

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NOTES ON THE BLISS METHOD FOR THE SEPARATION OF STRYCHNINE FROM QUININE.

BY L. E. WARREN AND A. H. CLARK.

A case having arisen in the experience of one of the writers (A. H. C.) in which an accurate separation of strychnine from quinine became desirable, the applicability of the method published by A. R. Bliss[1] was considered. As a preliminary, a mixture of quinine and strychnine in unknown proportions was tested by the method. The results obtained by the qualitative tests on the mixture were unsatisfactory. The fraction supposed to be quinine contained strychnine and the remaining fraction (supposed to be strychnine) contained quinine. Since the findings for the unknown mixture were so unsatisfactory, it seemed worth while to have the method checked on a known mixture of the two alkaloids.

In theory, the Bliss method is based primarily on the ready solubility of quinine in ether (1 in 1.5) and the scant solubility of strychnine in this solvent (1 in 5500). Secondarily it depends on the solubility of strychnine in water (1 in 6420). Although the solubility of strychnine is greater in ether than in water, the method requires a sufficient volume of water to dissolve all of the strychnine while permitting the use of but small volumes of ether. As recommended by Bliss, the total alkaloids are obtained in the usual way, weighed and dissolved in dilute sulphuric acid. An excess of water (more than 6500 times the weight of strychnine supposed

[1] A. R. Bliss, "A Method for Estimating Quinine and Strychnine when Occurring in Common Solution, JOUR. A. PH. A., 8, 804 (1919).

to be present) is added, the solution made alkaline with ammonia water, and the mixture shaken seven times with small portions of ether, using 35 Cc., 20 Cc., 10, 10, 10, 10 and 5 Cc., respectively. The combined ether solutions are washed with water, evaporated, the residue dried with the usual precautions and weighed as quinine. The ammoniacal liquid in the separator is then shaken with successive portions of chloroform, the solvent evaporated, the residue dried and weighed as strychnine. All of the determinations reported by Bliss were made on a mixture containing about thirty-two times as much quinine as strychnine, *i. e.*, determinations were not reported on mixtures containing other proportions of the two alkaloids.

A solution was prepared by dissolving 10.0286 Gm. of quinine and 0.9851 Gm. of strychnine in hydrochloric acid and diluting the solution to 500 Cc. with distilled water. Each Cc. of this solution contained 0.020057 Gm. of quinine (alkaloid) and 0.00197 Gm. of strychnine (alkaloid), the latter being present in approximately one-tenth of the concentration of the former. Two samples of 10 Cc. each from this solution were assayed strictly according to the method as published by Bliss. Sample A gave 0.2216 Gm. of alkaloid in the fraction supposed to be quinine and 0.0023 Gm. of alkaloid in the strychnine fraction. These values are equivalent, respectively, to 110.5 percent of theory for quinine and 11.7 percent of theory for strychnine. Sample B gave 0.2151 Gm. for the quinine residue and 0.0049 Gm. for the strychnine fraction, equivalent, respectively, to 107.3 percent of theory for quinine and 24.9 percent of theory for strychnine.

The residue from Sample A as obtained in the preceding paragraph, supposed to be quinine, was dissolved in a slight excess of sulphuric acid, a 5% solution of potassium ferrocyanide[1] added, the mixture agitated and allowed to stand over night. A noticeable precipitate formed. This was collected on a filter, the filter suspended in water, a slight excess of ammonia water added and the mixture shaken with chloroform until extraction was complete. The chloroform fractions were united, washed with a little water, the solvent evaporated, the residue dried and weighed. The residue weighed 0.0142 Gm. equivalent to 72.1 percent of the theoretical amount of strychnine originally taken in Sample A. This residue gave the "fading purple" test for strychnine, thus showing that the method of separation of the quinine from the strychnine as carried out by the Bliss method was not quantitative and that the ferrocyanide method was more nearly exact.

Another determination was carried out on 50 Cc. of the above-described alkaloidal solution (equivalent to 1.00286 Gm. of quinine and 0.09851 Gm. of strychnine). The results were 1.0708 Gm. for the quinine fraction, equivalent to 106.8 percent of theory, and 0.0274 Gm. for the strychnine fraction, equivalent to 27.8 percent of theory.

In order that the determinations might be made upon mixtures containing other proportions of quinine and strychnine than in those previously used, another solution was prepared by dissolving 3.0527 Gm. of quinine and 0.1964 Gm. of strychnine in dilute sulphuric acid and diluting the solution to 1000 Cc. Each Cc. of this solution contained 0.0030527 Gm. of quinine and 0.0001964 Gm. of strychnine. The concentration of quinine is approximately fifteen times that of the strychnine. Assays by the Bliss method were made upon 100 Cc. portions of

[1] C. Simmonds, *Analyst*, 39, 81 (1914).

this solution. Sample A gave 0.3191 Gm. for the quinine fraction, or 104.5 percent of theory and 0.0054 Gm. for the strychnine portion, or 27.5 percent of the amount taken. Sample B gave 0.3083 Gm. for the quinine fraction, or 101.0 percent of the quantity taken, and 0.0158 Gm. for the strychnine portion, or 80.4 percent of theory.

A solid mixture containing 81.52 percent of quinine and 18.48 percent of strychnine was prepared. This mixture was assayed by the Bliss method by each author independently. In one assay (A. H. C.) 0.0885 Gm. of the mixture (equivalent to 0.07215 Gm. of quinine and 0.01635 Gm. of strychnine) gave 0.0838 Gm. of extract supposed to be quinine and 0.0038 Gm. of extract supposed to be strychnine. The values are, respectively, 116.1 percent and 23.2 percent of theory. In another experiment 0.300 Gm. of the mixture was shaken once with 50 Cc. of ether. On evaporation the solvent gave 0.2560 Gm. of residue, equivalent to 85.3 percent of the total alkaloid taken or 104.7 percent of the quantity of quinine taken. This fraction contained an abundance of strychnine. No attempt was made to recover the strychnine by completing the second stage of the assay.

In another assay of the above-described solid mixture by the Bliss method (L. E. W.) 0.6032 Gm. of material, equivalent, respectively, to 0.49173 Gm. of quinine and 0.11147 Gm. of strychnine, gave, respectively, 0.5688 Gm. supposed to be quinine and 0.0318 Gm. supposed to be strychnine. These values are, respectively, 115.7 percent and 28.5 percent of the quantities taken. The quinine fraction contained an abundance of strychnine, as shown by the ferrocyanide and shakeout test already described. The strychnine fraction appeared to be practically free from quinine as shown by the very scant fluorescence of its solution in very dilute sulphuric acid.

Other assays by the Bliss method were made, using solutions as follows:

A solution containing 0.50248 Gm. of quinine and 0.01964 Gm. of strychnine was prepared. The quinine fraction weighed 0.5136 Gm. or 102.2 percent of theory. The strychnine fraction weighed 0.0067 Gm., or 34.1 percent of the quantity taken. Another solution containing 0.6139 Gm. of quinine and 0.01964 Gm. of strychnine was prepared. In this mixture the alkaloids are present in approximately the same proportions as in the solution assayed by Bliss. In the assay this solution gave 0.6222 Gm. for the quinine fraction and 0.0123 Gm. for the strychnine value. These findings are equivalent, respectively, to 101.3 percent and 62.6 percent of the quantities taken. The quinine fraction contained strychnine, as shown by the ferrocyanide test. For comparison the several findings are tabulated herewith.

It is believed that the proportions of quinine and strychnine used in these experiments have been varied sufficiently to be representative of conditions likely to be met with in the analysis of medicines. From the results obtained it appears evident that the method is unsuitable as a quantitative procedure for the separation of the two alkaloids. It does not compare favorably with the ferrocyanide method of separation. In general, the smaller the proportion of strychnine in the mixture the more nearly is the completeness of the separation.

It is known that the presence of large amounts of quinine in strychnine interferes[1] with the oxidation color reactions by which the latter alkaloid is identified.

[1] Allen, "Commercial Organic Analysis," 4th ed., p. 451.

By removing most of the quinine from mixtures of the two alkaloids, as is possible by a careful working of this process, the strychnine may be obtained in a state sufficiently pure for identification. The Bliss method, therefore, may be found useful in the qualitative analysis of medicines, although it appears to have no advantage over the well-known ferrocyanide method.

SEPARATION OF STRYCHNINE FROM QUININE.

BLISS METHOD.

	Weight taken.	Weight recovered.	Percentage recovered.	Remarks.
Quinine	0.20057	0.2216	110.5	contained strychnine
Strychnine	0.0197	0.0023	11.7	
Quinine	0.20057	0.2151	107.3	
Strychnine	0.0197	0.0049	24.9	
Quinine	1.00286	1.0708	106.8	contained strychnine
Strychnine	0.0985	0.0274	27.8	
Quinine	0.30527	0.3191	104.53	
Strychnine	0.01964	0.0054	27.5	
Quinine	0.30527	0.3083	101.0	
Strychnine	0.01964	0.0158	80.5	
Quinine	0.07215	0.0838	116.1	
Strychnine	0.01635	0.0038	23.2	contained quinine
Quinine	0.24456	0.2560	104.7	contained strychnine
Strychnine	0.05544	not recovered		
Quinine	0.49173	0.5688	115.7	contained strychnine
Strychnine	0.11147	0.0318	28.5	
Quinine	0.50248	0.5136	102.2	contained strychnine
Strychnine	0.01964	0.0067	34.1	free from quinine
Quinine	0.6139	0.6222	101.3	contained strychnine
Strychnine	0.01964	0.0123	62.6	

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THE ESTIMATION OF CRUDE FIBER IN GUM KARAYA.

BY E. H. GRANT.*

Gum Karaya, otherwise known in this country under the names of gum kadaya and Indian gum, and in India under many other names, is used very extensively as a thickening agent in foods and drugs; for various technical purposes, such as calico sizing, and in the manufacture of shoe polish.[1] Most of the gum is imported from Bombay, either directly or through the London markets, in bags holding approximately $1^1/_2$ cwt. (168 lbs.) each. During the year 1918 there were imported through New York a total of 1,260,000 pounds. It is carefully graded in India according to color and amount of foreign matter present. The principal impurity is the bark clinging to the gum. The presence of stones, especially those imbedded in the gum, is comparatively rare. This is quite in contrast to the lower grades of tragacanth which rarely contain bark in appreciable quantities, but very

* Work done while in charge of drug work, U. S. Food and Drug Inspection Laboratory, New York. Read before Cincinnati Section, A. Ph. A., October 1920.

[1] For discussion of the origin and uses of this gum, see "Karaya Gum, a Substitute for Tragacanth," Ewing, J. AMER. PHARM. ASSOC., 7, 787, 1918.