WILLARD DELL BIGELOW, 1866–1939

WILLARD DELL BIGELOW

Willard Dell Bigelow, twenty-fifth President of the Association of Official Agricultural Chemists, died on March 6, 1939. When this news was flashed through the press and scientific journals, not only his friends but all who knew his work in food chemistry and technology were deeply shocked. He was born at Gardner, Kansas, May 31, 1866. After completion of his High School studies, he attended Amherst College and was graduated in 1889. Then followed three years of graduate work and teaching at Oregon State College, Amherst, and Central High School, Washington.

On July 1, 1892, Dr. Bigelow entered the Bureau of Chemistry and began that long and brilliant service with Dr. Harvey W. Wiley in food chemistry, food technology, and food adulteration which culminated in the enactment of the Federal Food and Drugs Act of 1906. In the three years between 1904 and 1907 Dr. Bigelow was the author of no less than thirty bulletins and papers and the joint author of nine additional publications. This gives a partial picture of his almost ceaseless enterprise during this important period.

In 1913, Dr. Bigelow resigned as Assistant Chief of the Bureau of Chemistry to become director of the newly organized research laboratory of the National Canners Association. The work which he and his co-workers did stands out as pioneering in this specialized field of food technology, and it developed the fundamental scientific information on which practical technological operations could be predicated.

The first meeting of the Association of Official Agricultural Chemists that Dr. Bigelow attended was the tenth Annual Meeting, held in 1893. He served as a member of the Editing Committee in 1895 and of Committee C in 1904, as President in 1909 and as Editor and Secretary-Treasurer in 1912. He showed his interest in the work of this Association even after joining the National Canners Association, by attending all the annual meetings until the time of his death.

Dr. Bigelow had a personality that convinced a visitor at once of the strength of his convictions; he evidenced the true spirit of the scientist in that he knew his ground before he took a position or stand on any matter, and yet he was entirely free from bias and could clearly see the other angles or faces of any problem.

He was unusually willing to share with others the vast storehouse of knowledge gathered during his long experience in the fields of food chemistry, food technology, and food law enforcement. His unfailing kindness, his appreciation of the professional needs of less experienced men, and his readiness to offer practical suggestions on problems—all are attributes that stand out in the memory of those who were fortunate enough to have met and known him. One of his outstanding characteristics was his interest in the development and progress of younger men, to whom he was a source of stimulation and encouragement. The writer recalls many pleasant hours spent with Dr. Bigelow in which he pointed out with justifiable pride the progress made by many of the men who had been at one time or another associated with him.

Such a fine personality, such a keen grasp of the problems of technology, such a broad vision of science in relation to foods, their production and control, all combined in one man, make his loss to industry and to mankind truly a great one.

May his mantle of kindliness, ability, vision, and unselfishness fall on the shoulders of all of us who reaped the benefit of his wise experience, so that the words and deeds of Willard Dell Bigelow may guide us to lives of greater usefulness.

FRED C. BLANCK

SECOND DAY

TUESDAY—MORNING AND AFTERNOON SESSIONS—*Continued*

REPORT ON DRUGS

By L. E. Warren (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

Last year 23 topics were assigned to associate referees—4 less than in the previous year. Substantial progress was reported in 21 of these subjects. From the reports of the several associate referees the Referee recommends for tentative adoption quantitative methods for 14 drugs. These are acetophenetidin, caffeine and acetylsalicylic acid in mixtures, emulsion of cod liver oil, guaiacol, mandelic acid, theobromine in theocalcin tablets, ointment of mercuric nitrate, sulfanilamide, hypophosphites, hexylresorcinol, chlorobutanol, and aspirin and phenolphthalein in mixtures.

In addition, microchemical tests for 7 substances were recommended for adoption as tentative by the associate referees. These are berberine, cotarnine, diallyl barbituric acid, narceine, mandelic acid, narcotine and sulfanilamide. The Referee recommends the tentative adoption of methods for each of these drugs.

The Referee recommends closure of 12 topics.

One subject (nitroglycerin in mixtures) was discontinued without the adoption of a method. Ten topics, hypophosphites, hexylresorcinol, phenacetine, caffeine and aspirin, chlorobutanol, aspirin and phenolphthalein, emulsions of cod liver oil, citrine ointment, sulfanilamide, gums, and theobromine calcium tablets, were closed and methods tentatively adopted for each. One method (for guaiacol) was adopted as tentative and the topic reassigned for further study of guaiacol in mixtures.

New French Pharmacopoeia.—Since the last meeting the new edition of the French Pharmacopoeia has appeared. It became official April 1, 1938. The last edition appeared in 1908, but several supplements had been published at intervals since that time. This book is published in two volumes. The smaller (660 pp.) contains the French laws affecting pharmacy, the standard solutions, and general analytical reactions; the larger (1200 pp.) contains the usual information given in the text of pharmacopoeias but it is much more encyclopedic, i.e. has more of what would be expected in this country in the dispensatories. Among the new features are colored plates of many drug plants. About 1300 drugs are described, whereas the U.S.P. describes only 568.

New Book on Drug Analysis.—A review of Dr. Garrat's book, *Drugs and Galenicals: Their Quantitative Analysis*, was published in *This Journal*,

21, 517. This work is by an English author. It deals chiefly with B.P. and B.P.C. preparations; consequently the references are mostly to English authors and methods. By legal requirements drug analysts in this country must use the U.S.P. or the N.F. methods in the analysis of official drugs for legal investigations provided methods are supplied by these compendiums. However, since many B.P. and B.P.C. preparations have their analogues in the U.S.P. XI and N.F. VI, the methods which the author has found workable for the British products could be applied in most instances to such American preparations as are not provided with assays. Also they may be used as checks on the official methods.

Microchemical Tests for Alkaloids.—This topic has been under consideration for nearly a score of years and nearly all the important medicinal alkaloids have received attention. Altogether one or more tests have been adopted for each of 33 alkaloids. A few of these are of synthetic origin. This year berberine, cotarnine, narceine and narcotine were studied. Tests were developed for each by the associate referee and his collaborators.

The Referee concurs in all of the recommendations made by the associate referee.

Microchemical Tests for Synthetics.—Microchemical methods for the identification of synthetics were first studied by the A.O.A.C. in 1932. To date tests have been adopted for 19 synthetic drugs, a few of which are synthetic alkaloids. This year diallyl barbituric acid, mandelic acid and some of its salts, and sulfanilamide were studied. The Referee concurs in the recommendations of the associate referee and suggests that plasmochine also be studied.

Hypophosphites.—Last year the bromine oxidation method was studied. Commercial sirup of hypophosphites was used as test material but known samples were not assayed. This year the method was subjected to collaborative study. The standard solution of bromide-bromate used was not that of the U.S.P., N.F. VI, or the A.O.A.C. The results obtained are good. The associate referee recommends that the method be adopted as tentative. The Referee is of the opinion that an attempt should be made to make use of the A.O.A.C. standard solution instead of the more expensive solution recommended by Bruening, *J. Am. Pharm. Assoc.,* 25, 19, (1936). This phase of the subject was later brought to the attention of the associate referee. He made tests with the A.O.A.C. solution and found that the results were identical with those obtained with the more expensive solution. He then recommended that the A.O.A.C. solution be used. The Referee concurs.

Daphnia Methods.—The associate referee has continued his studies and has applied his method to tests for Vitamin E, certain alleged aphrodisiac drugs, cannabis, and numerous other toxic substances. The Referee recommends that the topic be continued.

Determination of Hexylresorcinol in Olive Oil.—Last year a method was

worked out which gave good results in the hands of three collaborators. Because of the limited amount of collaborative work done the topic was continued. This year but one collaborator worked on the problem. A specimen that gave 99.4 per cent (average) of recovery last year yielded 96.37 recovery after being kept for a year. Pure hexylresorcinol yielded 97.6 per cent recovery and a freshly prepared specimen in olive oil gave 97.8 per cent of recovery. The associate referee recommends that the topic be continued. The Referee believes that sufficient work has been done to warrant adoption of the method as tentative and so recommends.

Nitroglycerin.—The associate referee worked faithfully, but the results this year were as disappointing as they have been for the past two years. The associate referee recommends that the subject be discontinued temporarily. The Referee concurs.

Guaiacol.—Last year a method in the literature, which depends on the determination of the alkoxyl group, was adapted to the determination of guaiacol by the associate referee, *This Journal,* **21,** 543. This year the method was subjected to collaborative tests on known materials. The results are good. The associate referee recommends that the alkoxyl method be adopted as tentative. The Referee concurs.

Biological Testing.—No report was received from the associate referee. The Referee recommends that the subject be continued.

Iodine Ointment.—This topic has been under investigation for several years. The associate referee first developed a method for the determination of total iodine. This was included in the U.S.P. XI so that it was not adopted by the Association. Last year a method for the determination of free iodine was adopted tentatively, but with the provision that it be not advanced to official status, presumably until the subject could be closed. This year the associate referee and his collaborators studied methods for the determination of organically combined iodine. A method was developed which gave good results in a collaborative way on freshly made ointment but which failed on old specimens.

The Referee concurs in the recommendations of the associate referee.

Separation of Acetphenetidin, Acetylsalicylic Acid, and Caffeine.—This topic has been under investigation for several years. This year the associate referee applied a modification of the method previously tried. The recoveries of the three medicinal agents were as close to theory as could be expected in such a difficult separation. The Referee concurs in the recommendations of the associate referee.

Gums.—For five years the associate referee has attempted to identify various gums by precipitation methods. He has now applied various reactions to the precipitates and has developed tests which collaborative work indicates to be effective. The Referee concurs in the recommendations of the associate referee.

Theobromine Calcium Tablets.—This topic has been studied for two

years. This year the tentative A.O.A.C. method, *Methods of Analysis*, 1935, 590, was compared with the Boie process, *C.A.*, **25**, 169 (1931). The results from a limited amount of collaborative work are good. The associate referee recommends the deletion of the present tentative method and the substitution therefor of the Boie procedure. The Referee recommends that the method studied by the associate referee and his collaborators this year be adopted as a tentative alternative method, and that the status of the present tentative method remain unchanged.

Chlorobutanol.—Last year the associate referee applied the distillation method (with subsequent conversion to chloride) to the determination of chlorobutanol in mixtures. The results were not entirely satisfactory. This year the work was continued, and the collaborative findings are satisfactory. The Referee concurs in the recommendations of the associate referee.

Aspirin and Phenolphthalein Mixtures.—This is the fourth year that this topic has been studied. The Hitchens method, *J. Am. Pharm. Assoc.*, **23**, 1084 (1934), modified, was subjected to collaborative study. The results are moderately good. The Referee concurs in the recommendation that the method be adopted as a tentative method, and recommends that the topic be closed.

Aminopyrine and Phenobarbital in Mixtures.—Last year the associate referee devised an empirical method for determining each of these substances in admixture with each other but no collaborative work was done. This year the method was subjected to collaborative study on mixtures of the two drugs without excipient. The results are moderately good. The Referee concurs in the recommendation of the associate referee.

Elixir of Terpin Hydrate and Codeine.—The Association has adopted a method for the assay of terpin hydrate in the elixir (without codeine). Last year the associate referee and his collaborators developed an empirical method for determining both the terpin hydrate and the alkaloid. The results are reasonably good for codeine but are not entirely satisfactory for terpin hydrate. This year essentially the same method was tried again. The results are still not completely satisfactory for the recovery of terpin hydrate. The associate referee is aware of the inherent faults of the method but recommends its tentative adoption in the absence of a more accurate procedure. The Referee concurs.

A member of the Association criticizes the proposed method because of the small sample taken—only about 3 cc. of 0.02 *N* acid being required to titrate the codeine. For the alkaloid he uses a 50 cc. sample, dilutes with 50 cc. of water, and applies the double shake-out procedure. A little terpin hydrate appears with the alkaloid but this does not interfere with the titration. About 15 cc. of 0.02 *N* acid is required.

Emulsions.—This is the second year that this topic has been studied. Last year the associate referee tried various methods of extracting the oil

from emulsion of cod liver oil, but no collaborative work was done. This year a method was developed which gave satisfactory collaborative tests. Although the number of collaborators was not large and the method was not tried on commercial products, the associate referee recommends that the method be adopted as a tentative procedure.

Ointment of Mercuric Nitrate (*Citrine Ointment*).—This is the second year of study for this product. The associate referee and his collaborators used a modification of the method employed last year, *This Journal*, **21**, 579. Good results were obtained. The Referee concurs with the associate referee's recommendation that the method be adopted.

Rhubarb and Rhaponticum.—No work was done. Rhaponticum is refused entry into the United States, so it was necessary to obtain a special permit to allow entry of specimens for experimental purposes. The topic should be continued.

Theophylline Sodium Salicylate.—Last year methods were developed for determining theophylline and salicylic acid, but the results were not considered sufficiently consistent for adoption of the methods. This year but little work was done. The Referee recommends that the topic be continued.

Sulfanilamide.—The associate referee tried several methods, of which one was selected. The Referee concurs with the recommendations of the associate referee that the method be adopted as tentative.

Mandelic Acid.—This is a new topic. The associate referee devised two qualitative tests for the acid. Some of the collaborators have questioned whether these tests are sufficiently characteristic for adoption. The quantitative tests consisted of shaking out a diluted, acidified solution with a mixture of chloroform and ether (2+1) and titrating the residue after careful removal of the solvent. The results are good.

The Referee questions whether the qualitative tests are sufficiently specific, therefore he concurs only in the recommendation that the quantitative assay be adopted as tentative.

REVISION OF METHODS

During the summer the Referee sent a circular letter to each of the associate referees and to certain of the former associate referees, which read in part as follows:

> Owing to the important changes in the by-laws of the constitution of the Association, adopted at the last meeting, *This Journal*, **21**, 101, it becomes more important than formerly that studies be made early concerning any possible changes in tentative or official methods already adopted. Two years (two readings) are required before a tentative method may be made official, final action, and additional collaborative work is expected although not obligatory. The same conditions apply to the deletion or amendment of an official method. It would seem desirable, therefore, at this time to make an editorial survey of the methods for drugs in *Methods of Analysis* and of those adopted since the book was revised, in order to determine whether any changes should be advised.

Each associate referee who is responsible for one or more methods in the drug section of the A.O.A.C., which are not now official, final action, is requested to make a critical editorial study of such methods and to report to the next meeting. This report might include one or more of the following:

(1) A recommendation as to whether the method should be deleted, with reasons.

(2) A recommendation as to whether the method should be retained as tentative, with reasons.

(3) A statement as to whether the method should be advanced from its present status (tentative or official, first action) to the status next higher (official, first action, or official, final action).

(4) Such other recommendation as in the opinion of the associate referee should be made.

Chloroform in Mixtures.—This method was developed chiefly to determine small quantities of chloroform in cough sirups. It consists in distilling the chloroform from a neutral solution in presence of alcohol into concentrated alkali and determining the resultant chloride. The method was adopted in 1931, although it was known for some years before that.[1]

Several minor criticisms concerning the method have been received. One is that low results are obtained. Roberts and Murray,[2] who first described the method in detail, obtained about 98 per cent recovery in a number of trials. Another criticism is that bumping results when the full amount of calcium carbonate (1 g.) is used. Bromides, chlorides, and iodides may be present in cough sirups, and the calcium carbonate is added to prevent the liberation of volatile mineral acids. Doubtless the quantity of calcium carbonate could be reduced although Putt (*loc. cit.*) recommended this amount. The Referee is of the opinion that dilution with water before distillation to prevent precipitation of sugar will overcome the bumping. The addition of glass beads should accomplish the result. The several criticisms received were submitted to each of the former associate referees on the subject, but owing to resignations and reassignments three associate referees had worked on this topic before the method was finally adopted. These chemists recommended that the subject be studied again by the A.O.A.C.

Associate Referee Moraw recommended that the words, "citrate bottle," in paragraph **105** be replaced by the words, "pressure bottle fitted with a rubber gasket that will provide a tight seal." He also recommended that the following caution be inserted in an appropriate place:

Caution.—Do not cool the pressure bottle suddenly. It is best to allow it to cool in the water in which it was boiled.

Associate Referee Kunke recommended the deletion of one of the reagent alcoholic potassium hydroxide solutions, **104** (a), on the ground that the stronger one, **106** (a) is sufficient.

The Referee objected to the expression " . . . fitted with a rubber gas-

[1] Putt, *Am. Food J.*, 10, 467 (1915); Lyons, Analysis of Drugs, ed. 2, p. 309 (1920).
[2] *Am. J. Pharm.*, 101, 654 (1930).

ket that will provide a tight seal," on the ground that some pressure bottles are provided with ground-glass plates, which are held in position by suitable screws.

In view of the criticisms offered and also the recommendations of the earlier associate referees, the Referee requested the last associate referee to make some additional trials. He has done this and has submitted a special report. He and his collaborators find that the tentative method yields about 98 per cent of the chloroform added. He recommends several minor amendments in the method, and the Referee concurs in these recommendations.

Aloin.—This method has been in the tentative stage since 1932, and no adverse comments have been received. The associate referee recommends that the method be advanced to "official, first action." The Referee concurs.

Bismuth Compounds in Tablets.—The associate referee recommends that this tentative method, *Methods of Analysis, A.O.A.C.*, 1935, 592, be amended by inserting before the beginning of the present text the following paragraph:

Count and weigh a suitable number of tablets and ascertain their average weight. Pulverize the tablets and preserve the powder in a tightly stoppered bottle.

He also recommends that the method be retained in a tentative state for the present. The Referee concurs in both recommendations.

Camphor.—This method, which depends on polarimetry, has been official for some years, *Methods of Analysis, A.O.A.C.*, 1935, 560, 51. The associate referee recommends its deletion on the ground that synthetic camphor may not be determined by it. In view of the fact that the dinitrophenyl hydrazine method has not given entirely satisfactory results the Referee recommends that no changes be made in the status of the A.O.A.C. method at this time.

The Referee further recommends that the statement, "Not applicable to synthetic camphor," be inserted in parentheses between the title and the text of the article.

Cascara Sagrada.—The tentative method for cascara sagrada, *Methods of Analysis, A.O.A.C.*, 1935, 583, has been criticized in that it requires too much time of the analyst. It has also been questioned whether the results obtained in the assay represent therapeutic value. One member of the Association believes that the method should be deleted. The associate referee is of the opinion that further work should be done. In view of these criticisms and the fact that no further collaborative work has been carried out, the Referee recommends that no changes in status be made at this time.

Cinchophen in Presence of Salicylates.—A method for this determination was studied last year. It was essentially the method devised by Emery.[1]

[1] *J. Am. Pharm. Assoc.*, 17, 18 (1928).

After suitable collaborative work had been carried out with good results the method was adopted as tentative. No criticisms having been received, the associate referee now recommends that the tentative method for cinchophen in presence of salicylates be advanced to official, first action. The Referee concurs.

Cocaine.—Two methods for the determination of cocaine have been adopted as tentative, *Methods of Analysis, A.O.A.C.*, 576. In the first the alkaloid is released by sodium bicarbonate, the alkaloid removed by ether, and the residue titrated. In the other the alkaloid is removed by petroleum benzin and the titration completed as usual. Provision is made for checking by conversion to benzoic acid and determining that. The associate referee recommends the deletion of the first method (96) because of the lack of originality. He recommends further the advancement of the second method to "official, first action."

The Referee therefore recommends that Method I (Section 96) be deleted, first action, and that Method II be advanced to official, first action.

Dinitrophenol (or its Sodium Compound).—A method for the determination of dinitrophenol was adopted tentatively in 1936, *This Journal*, 20, 82. No adverse criticisms having been received the associate referee recommends that the present tentative method be made official, first action. The Referee concurs.

Ether.—A method for the determination of ether was adopted as tentative in 1932, *Methods of Analysis, A.O.A.C.*, 1935, 584. The associate referee reports that no adverse criticisms have been received and he recommends that the method be adopted as official, first action. The Referee concurs.

Homatropine in Tablets.—A method for the assay of this preparation was adopted as tentative after two years' study by the associate referee and his collaborators. The method is the well known "double shake out" procedure. No criticisms of the method having been received, the associate referee recommends that the method be advanced to official, first action. The Referee concurs.

Microchemical Tests for Synthetics.—In accordance with the policy of the Association to reduce the number of reagents as much as possible the associate referee recommends:

(1) That the directions for preparing bromide-bromate solution, 180 (b) be deleted and the following statement substituted: "Prepare as directed under 26 (c)."

(2) That the directions for preparing magnesia mixture, *Ibid.* (h), be deleted and the expression, "Prepare as directed under II, 7 (c)," be substituted.

(3) That the directions for preparing gold chloride solution, *Ibid.* (i), be deleted and the expression, "Prepare as directed under 176 (j)," be substituted.

(4) That the directions for preparing Kraut's reagent, *Ibid.* (m), be deleted and the expression, "Prepare as directed under 176 (b)," be substituted.

The Referee concurs in each of these recommendations.

Iodoform and Iodoform Gauze.—Methods for the assay of these preparations were adopted as tentative in 1931, *This Journal,* 15, 85. The Referee reports that no adverse criticisms have been received. He recommends that the status be advanced to official, first action. The Referee concurs.

Ipecac and Opium Powder (*Dover's Powder*).—This preparation is described in the U.S.P. XI, but no assay is provided. Other preparations of ipecac and opium, such as the tincture and tablets, are on the market, and a method for the assay of morphine in these products was published in 1934. This method has recently been modified.[1] The test depends on the formation of nitroso-morphine with nitrites in acid solutions and colorimetric comparison of this in ammoniacal solution with knowns. It is recommended that assay methods for ipecac and opium powder be studied.

Methylthionine chloride (*methylene blue*).—A tentative method for the assay of methylene blue was adopted by the Association in 1923, *This Journal,* 7, 20 (1923). Later this became official, *Ibid.,* 10, 69 (1927). This is an iodometric titration in the presence of acetic acid. It provides also for the determination of the drug in oil mixtures, solutions, etc. The U.S.P. XI adopted an iodometric method also, but since this does not provide for the separation of the drug from mixtures, the A.O.A.C. method was retained.

Methods for the assay of methylene blue have been studied in a collaborative way by a subcommittee sponsored by the American Drug Manufacturers Association and the American Pharmaceutical Manufacturers Association. This subcommittee found that the perchlorate method originally devised by Deahl and Maurina gave satisfactory results in collaborative trials. Essentially the method consists in precipitating an aqueous solution of methylene blue with an aqueous solution of potassium perchlorate and weighing the dried compound. The method was claimed by the subcommittee to be superior to the U.S.P. method (and presumably A.O.A.C. method). This information was brought to the attention of the associate referee, who made a preliminary study of the subject. He rejected the perchlorate method in favor of the A.O.A.C. and the U.S.P. XI methods. In view of his brief report the Referee recommends that no action be taken at this time.

Monobromated Camphor.—Two methods have been adopted for the assay of this product, *Methods of Analysis, A.O.A.C.,* 1935, 561. Method I is official and the other method is tentative. One converts the organically combined chlorine to chloride by sodium amalgam and the other uses

[1] *Quart. J. Pharm. Pharmacol.,* 10 468 (1937).

alcoholic alkali for the purpose. The associate referee is of the opinion that the second of these methods might be deleted. The Referee is of the opinion that each should be retained in its present status and so recommends.

Barbital and Phenobarbital.—The associate referee recommends that the official method, *Methods of Analysis, A.O.A.C.*, 1935, 582, **112**, be amended, first action, by inserting at the end of paragraph **112** the following expression: "Determine the melting point to check the purity of the residue." The Referee concurs.

The associate referee also recommends that the tentative alternative method for barbital and phenobarbital (applicable in presence of stearic acid), *Ibid.*, **113**, 582, be advanced to official, first action. The Referee concurs.

Sabadilla.—Methods for the assay of this drug were studied in 1929, *This Journal,* **12**, 305. At that time the associate referee recommended that the method subjected to collaborative study be adopted as tentative. This was not done on the ground, *Ibid.*, 77, that the method was essentially a U.S.P. X procedure. The associate referee now recommends that the method then proposed be adopted as tentative. He points out that no official compendium in the United States has a method for the assay of sabadilla. The Referee has studied the literature since 1929. Gsterner recommends a method which is considerably different from that proposed by the associate referee. In view of these facts the Referee recommends that the subject be reassigned for 1939.

Pilocarpine Hydrochloride.—This salt is described in N.F. VI, but no assay is provided. The associate referee reports that he has had no criticisms of the method except the "rather indefinite parenthetical statement in regard to the sharpness of the end point." In view of the fact that he makes no recommendation the Referee believes that the present status of the method should not be advanced.

Phenolsulfonates.—A method was adopted as tentative in 1933. The associate referee has reviewed the subject and recommends that this method be retained in its present status. The Referee concurs.

Potassium Thiocyanate vs. Ammonium Thiocyanate as Reagent in Microchemical Tests.—In several instances potassium thiocyanate is used as a microchemical reagent in the identification of alkaloids and synthetics. In others the ammonium salt is employed. Owing to the greater availability of the ammonium salt, studies were undertaken by each of the associate referees to ascertain whether the ammonium salt could be used interchangeably with the potassium salt in the microchemical tests already adopted. Each associate referee reported that the reactions took place equally well with the ammonium salt. Therefore it is recommended—

(1) That the words "potassium thiocyanate" in the 15th line of par. 180, p. 605, be changed to read "Ammonium Thiocyanate."

(2) That the reagents under "Neocinchophen" and "Pyridium" on p. 606 be changed from "potassium thiocyanate" to read "ammonium thiocyanate."

(3) That the reagent for ethylhydrocupreine, "potassium thiocyanate," *This Journal*, **20,** 81 (1937), be changed to read "ammonium thiocyanate."

Thymol.—The Referee concurs in the associate referee's recommendation that this method be advanced to official, final action.

Thymol in Antiseptics.—The Referee concurs in the associate referee's recommendation that this topic be advanced to official, first action.

Wagner's reagent.—This reagent is described three times in the section on drugs in *Methods of Analysis* and each formula is different. Furthermore, none is exactly the same as the original formula described by Wagner in 1863. However, one is a close approximation.

The associate referee recommends that the directions for preparing Wagner's reagent, **180 (d),** be deleted and the statement, "Prepare as in **176 (c),**" be substituted. He further recommends that the direction in **5,** p. 543, be deleted and the expression, "Prepare as directed in **176 (c),**" be substituted. The Referee concurs.

Morphine in Sirups.—No adverse comments have been received for this method but the associate referee made no recommendation concerning it. The Referee recommends that the status be advanced to official, first action.

NEW SUBJECTS

Ointment of Yellow Mercuric Oxide.—The U.S.P. XI describes the preparation of ointment of yellow mercuric oxide and provides an assay for the product. An alternative method is desired for checking purposes. It is recommended that an associate referee be appointed to study this topic.

Aspirin, Phenacetin, and Salol.—Tablets containing mixtures of these three ingredients are on the market. The A.O.A.C. has adopted no method for the separation of these constituents in mixtures. It is recommended that this subject be studied.

Arecoline hydrobromide.—This salt is being used by veterinarians. It is described in N.F. VI, but no assay is provided. It is recommended that arecoline hydrobromide be studied with particular reference to the assay of the tablets.

Benzedrine.—This synthetic has come into considerable use in the last two or three years, but the Association has adopted no tests for it. It is recommended that benzedrine sulfate be studied.

Hydroxyquinoline Sulfate.—Hydroxyquinoline sulfate is marketed in various mixtures. The Association has adopted a microchemical test for its identification but none for its determination. It is recommended that chemical methods for the determination of hydroxyquinoline sulfate be studied.

Physostigmine Salicylate.—Tablets of physostigmine salicylate are marketed for use in ophthalmology, but no assay has been adopted by the A.O.A.C. It is recommended that assay methods for physostigmine salicylate tablets be studied.

Plasmochine.—The synthetic known as plasmochine has come into considerable use as an antimalarial remedy, particularly in mixtures with quinine. No microchemical tests for its identification have been adopted by the A.O.A.C. It is recommended that microchemical tests for plasmochine be studied, and that it be assigned for study for chemical methods of assay.

Separation of Acetanilid and Salol.—A problem arose involving the separation of acetanilid and salol. The A.O.A.C. has adopted two methods for separating phenacetin and salol, one of which has been included in N.F. VI. These methods were devised by Emery, Spencer, and LeFebre, but no process for separating acetanilid and salol was reported.[1] Both of the A.O.A.C. methods for phenacetin and salol were applied by several members of the Association to a specimen of commercial tablets of acetanilid and salol. Neither method gave consistent results. Several attempts to separate known mixtures of acetanilid and salol by the same methods failed to give satisfactory results. The Referee has used the Salkover[2] method with satisfactory results.

It is recommended that an associate referee on acetanilid and salol be appointed.

REPORT ON MICROCHEMICAL TESTS FOR ALKALOIDS

By C. K. Glycart (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Continuing the subject this year, the Associate Referee made tests for berberine, cotarnine, narceine, and narcotine. Narceine, an alkaloid of opium, is characterized by its unusual property to form crystals, which are blue in color with Wagner's reagent, zinc chlor-iodide, and other iodized reagents. Stephenson[3] states that platinic chloride is the second best test for narceine and that beautiful feathery rosettes are formed from the amorphous precipitates in all solutions.

Narcotine, also an alkaloid of opium, forms crystalline precipitates in rosettes of needles with the reagent potassium hydroxide. Practically the same results are obtained with potassium acetate, sodium carbonate, and sodium phosphate.

Cotarnine, a derivative of opium, is recognized in the National Formulary as Cotarnine Chloride and is commercially known as "Stypticin." It is a yellow powder. According to Amelink,[4] potassium ferrocyanide

[1] *J. Ind. Eng. Chem.*, 7, 681 (1915).
[2] *Am. J. Pharm.*, 88, 484 (1916).
[3] Microchemical Tests for Alkaloids, p. 58 (1921).
[4] Schema zur Microchemischen Identifikation von Alkaloiden (1934).

forms characteristic crystals with cotarnine when acidified with hydrochloric acid.

In the preliminary work last year, the tests for berberine were considered insufficient for identification. In the work this year, clusters of radiating needles developed slowly with mercuric chloride if an excess of the reagent was avoided; crystals were readily obtained by the hydrochloric acid reagent with a saturated solution of berberine hydrochloride.

Directions for the tests, control specimens consisting of berberine hydrochloride, cotarnine chloride (N.F.), narcotine sulfate, and narceine hydrochloride, also samples for identification marked Nos. 1, 2, 3, and 4, were sent to the collaborators. The material for the controls was considered sufficiently pure for the work.

The methods were published in *This Journal*, **22**, 88 (1939).

COLLABORATORS RESULTS AND COMMENTS

All the collaborators reported the correct identifications, namely, No. 1, Narceine; No. 2, Narcotine; No. 3, Cotarnine; No. 4, Berberine.

They commented as follows:

John R. Matchett, Bureau of Narcotics, Washington.—

Berberine.—It was found that a 1:100 solution of berberine hydrochloride could only be prepared in hot water and that crystals separated on cooling. Upon adding a drop of the hydrochloric acid reagent to a 1:400 solution or 1:200 solution, very fine needles formed immediately, and the number increased gradually as the drop stood. The needles formed with hydrochloric acid appeared to be quite distinctive, but crystals of a definite complex compound would seem to be preferable for identification to crystals of the alkaloid hydrochloride itself.

The $HgCl_2$ reagent produced an amorphous precipitate in the 1:200 solution and the 1:400 solution, but no crystals were formed even after standing one-half hour.

Cotarnine.—With the platinic chloride reagent a 1:200 solution of cotarnine hydrochloride gave large yellow sheaves of long needles along with long, very slightly curved, colorless, or faintly yellow needles. With a 1:200 solution the $HgCl_2$ reagent gave a precipitate, amorphous at first, quickly going over to rapidly growing long colorless needles. With a 1:200 solution the $K_4Fe(CN)_6$ reagent used as directed gave crystals as described, forming very slowly at the edge of the drop. With a 1:50 solution similar crystals formed much more rapidly. Of the tests proposed for cotarnine, the platinic chloride reagent appears best. The others, however, appear to be satisfactory except that with the $K_4Fe(CN)_6$ reagent crystal formation is exceedingly slow, especially in the more dilute solution.

Narceine.—With a 1:200 solution the platinic chloride reagent produced crystals as described, which formed slowly. There was no preliminary amorphous precipitate. With a 1:200 solution Wagner's reagent produced a thick mat of interlaced needles and burrs, forming first on the edge of the drop. They were so dark as to appear black rather than blue.

Narcotine.—With a 1:200 solution the KOH reagent produced crystals as described.

Irwin S. Shupe, U. S. Food and Drug Adm., Kansas City.—Merck's Index gives the solubility of berberine hydrochloride as 1 to 400. A saturated solution was satisfactory for both $HgCl_2$ and HCl tests. The 1:200 concentration of cotarnine seems

to be the best for all three tests for this alkaloid. Cotarnine ferrocyanide is especially characteristic. The narceine hydrochloride was not sufficiently soluble to make a 1:200 solution unless a small excess of HCl was present. Both a saturated solution and a 1 to 200 with excess HCl were satisfactory. The rosettes of needles described for narcotine formed more readily if the test drop was stirred slightly. (10% NH_4OH appeared to give the test more readily than KOH.)

J. H. Cannon, U. S. Food and Drug Adm., St. Louis.—The test for narcotine with KOH is not so quickly obtained as are the others, but good crystals were obtained on long standing. I did get crystals with narceine that were really blue this time.

F. K. Ballard, U. S. Customs Laboratory, Chicago.—

Berberine.—With $HgCl_2$. One part of berberine hydrochloride did not dissolve completely in 100 parts of water. Solution was filtered and filtrate used for test. Results seemed somewhat better when this solution was diluted with an equal amount of water. With 5% HCl the results were as given in the method.

Cotarnine.—With H_2PtCl_6 1:200. Results as given in method. With $HgCl_2$ long branching needles as stated in method were observed, but the color appeared to be pale yellow. Chains of rhombic plates and tree-like forms were also observed. With $K_4Fe(CN)_6$ acidified with 1 drop of 5% HCl, the results were as given in the method.

Narceine.—With H_2PtCl_6. One part of narceine hydrochloride did not dissolve completely in 100 parts of water. The solution was filtered and the filtrate was used for this and the following test. Results were as given in method. The color of the rosettes was yellow, and several minutes were required for formation of rosettes. With Wagner's reagent a saturated solution of narceine hydrochloride was used. The results were as stated in the method.

Narcotine.—With KOH 1:200 the results were as stated in the method.

W. J. Rice of the Eli Lilly and Company, Chemical Control Laboratories, Indianapolis.—All these tests responded satisfactorily although it will be noted that we also observed brown plate-like octagons among the burr-shaped crystals in the $K_4Fe(CN)_6$ test for cotarnine.

Charles C. Fulton, Internal Revenue Service, St. Paul.—

Narceine.—Zinc KI_3[1] is strongly recommended for the blue needles. If traces of alkaloidal impurities are present in the narceine it is generally quite impossible to obtain these crystals with Wagner's reagent, and even with pure narceine Wagner's reagent will not crystallize so readily as zinc KI. Note that this reagent is *not* Stephenson's "zinc chlor-iodide," for which he gave a formula containing "free" iodine, and therefore having the same defects, as a narceine reagent, as Wagner's reagent. With platinum chloride—crystallized readily in rosettes, rather dense and dark—the crystals seemed to be poorly formed prisms rather than feathery forms when examined under high power.

I should rate the blue needles with zinc KI as easily the best test. The narceine solution need not be any particular strength; crystals are obtainable readily down to about 1:1600.

The arseni-molybdic acid reagent is sensitive to about 1:3300, and a dilute solution is recommended for the test. I am inclined to consider it the second best test; H_2PtCl_6 might come in as the third best test. It is less sensitive.

Narcotine.—The only test I know of at present is the crystallization of the free base. Any basic reagent will do, narcotine being such a weak base that it is thrown out even by $KC_2H_3O_2$. Larger crystals, prisms, can be obtained by precipitating from distinctly acid solution by concentrated $KC_2H_3O_2$. I think NH_4OH, or Na_3PO_4, or the like, may give a little better crystallization and a more sensitive test than potassium hydroxide; however, the test is about the same.

[1] Charles C. Fulton, *Am. J. Pharm.*, **104**, 244 (1932).

Cotarnine.—This alkaloid gives a large number of crystals. I would not attempt to say at present whether the three tests you give are better or as good as any others available; however, all three are good.

Berberine.—As Stephenson remarks, a variety of reagents give very similar crystals. The test with 5% HCl is no doubt highly characteristic.

SUMMARY

The alkaloids were identified correctly by all the collaborators. In regard to the test for berberine, by mercuric chloride, Matchett reported that no crystals were found even after standing one-half hour. In the test for cotarnine with potassium ferrocyanide, Rice reported that amber-brown plates were observed among the burr-shaped crystals. In the test for narcotine, Shupe and Fulton stated that ammonium hydroxide is more sensitive than potassium hydroxide. Fulton recommended zinc potassium iodide for the blue needles in preference to Wagner's reagent for narceine.

These tests were repeated by the Associate Referee. Mercuric chloride with a 1:400 solution of berberine produced an amorphous precipitate, as correctly stated by Matchett. With a 1:800 solution, however, needles were formed. The test is not sufficiently distinctive and further study should be made.

The tests were modified to include the observations made by the collaborators.

Material for tests for coniine and cytisine were not available.

This year, the Referee on Drugs suggested that stovaine be studied. This will be included next year.

RECOMMENDATIONS[1]

It is recommended—

(1) That the microchemical tests for cotarnine, narceine, and narcotine be made tentative.

(2) That the test for berberine by hydrochloric acid reagent be made tentative.

(3) That further tests for berberine be studied.

(4) That coniine, cytisine, stovaine, and phenacaine (holocaine) be studied.

REPORT ON MICROCHEMICAL METHODS FOR SYNTHETICS

By IRWIN S. SHUPE (U. S. Food and Drug Administration, Kansas City, Mo.), *Associate Referee*

This seventh report on the subject of Microchemical Methods for Synthetics describes a study of tests for mandelic acid, sulfanilamide (p-aminobenzenesulfonamide), and diallylbarbituric acid.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 56 (1939).

A study of the microchemical reactions of mandelic acid and sulfanilamide was made by James W. Mitchell and reported in his unpublished thesis.[1] He shows that for sulfanilamide the following reagents produce crystalline precipitates: platinic chloride, picric acid, picrolonic acid, chromic acid, bromine water, and sodium nitrite. He also obtained characteristic reactions by diazotization and coupling with phenol and by precipitation of free sulfanilamide from acid solutions with sodium carbonate and other alkaline salts. For mandelic acid he obtained crystalline precipitates with zinc sulfate, cadmium chloride, mercuric chloride, mercurous nitrate, stannous chloride, copper sulfate, lead nitrate, silver nitrate, and potassium ferrocyanide.

Characteristics of synthetics studied

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Diallyl-barbituric acid	Dry powder	—	Lead tri-ethanol-amine	Stir a small amount of the synthetic into a drop of the reagent. Rods singly and in clusters.
	Dry powder	—	Barium hydroxide	Stir a small amount of the synthetic into a drop of the reagent. Rods singly and in groups.
Mandelic acid	Water	1–100	Lead acetate	Rosettes of thin curving plates.
	Water	1–100	Mercurous nitrate	Burr-shaped groups of needles.
Sulfanilamide	Dry powder	—	Benzaldehyde	Stir thoroughly a small amount of synthetic into a drop of regent. 4-sided plates.
	0.1 *N* HCl	Saturated solution	Sodium nitrite	Yellow needles.

Yakowitz, *This Journal*, **21**, 351, described microchemical tests for sulfanilamide with benzaldehyde and cinnamic aldehyde. Benzaldehyde and sodium nitrite were considered especially suitable as reagents for sulfanilamide. Lead acetate and mercurous nitrate were chosen as the best reagents for mandelic acid.

[1] Thesis in partial fulfillment of Degree of Bachelor of Science in Chemistry, June ,1938, Philadelphia College of Pharmacy and Science.

According to Itallie and Steenhauer,[1] diallylbarbituric acid unlike most other barbiturates forms a sparingly soluble crystalline salt with barium hydroxide. It also forms a crystalline precipitate with a solution of lead acetate containing triethanolamine.

The synthetics used in these tests complied with the N.N.R. standards for identity for diallybarbituric acid,[2] and for sulfanilamide.[3] The mandelic acid used was the racemic form with a melting point of 118°–119° C. Directions for the tests, control specimens of the synthetics, and unknown samples for identification were sent to collaborators.

The unknown samples were: No. 1, diallylbarbituric acid; No. 2, sulfanilamide; No. 3, mandelic acid.

The method was published in *This Journal*, **22**, 89 (1939).

RESULTS OF COLLABORATORS

All the collaborators reported the correct identifications, namely No. 1, diallylbarbituric acid; No. 2, sulfanilamide; and No. 3, mandelic acid.

The collaborators commented as follows:

J. H. Cannon, U. S. Food and Drug Adm., Chicago.—I have no adverse comments to make regarding these tests. For diallylbarbituric acid, barium hydroxide seems somewhat better than lead triethanolamine in that crystal formation is more rapid and the crystals seem to grow to a larger size.

W. J. McCarthy, U. S. Food and Drug Adm., St. Louis.—By following your directions the identifications were checked without much difficulty.

M. L. Yakowitz, U. S. Food and Drug Adm., San Francisco.—All of the tests responded nicely except the barium hydroxide test for diallylbarbituric acid. I repeated the test several times but was unable to achieve satisfactory results.

H. R. Bond, U. S. Food and Drug. Adm., Chicago.—The reagents formed crystal structures easily identified through the controls.

DISCUSSION

The unknown samples were correctly identified by each of the collaborators. Difficulty in the test for diallylbarbituric acid with barium hydroxide may be due to using too small an amount of the powdered synthetic in the test drop. The tests described are considered satisfactory for the identification of diallylbarbituric acid, mandelic acid, and sulfanilamide.

RECOMMENDATIONS[4]

It is recommended—

(1) That the microchemical methods presented for diallylbarbituric acid, mandelic acid, and sulfanilamide be adopted as tentative.

(2) That other important synthetics be studied such as para-phenylenediamine and para-toluenediamine.

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[1] Microchemie, Emich Festschrift, 1930, 166.
[2] New and Non-Official Remedies, 1937, p. 103.
[3] *J. Am. Med. Assoc.*, **109**, 358 (1937).
[4] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 56 (1939).

(3) That the directions for preparing bromide-bromate solution, *Methods of Analysis*, A.O.A.C., 1935, **XXXIX, 180 (b)**, be deleted, and the following statement substituted: "Prepare as directed under **XXXIX, 26, (c)**."

(4) That the directions for preparing magnesia mixture, *Ibid.*, **(h)**, be deleted and the expression, "Prepare as directed under **II, 7 (c)**," be substituted.

(5) That the directions for preparing gold chloride solution, *Ibid.*, **(i)**, be deleted and the expression, "Prepare as directed under **176 (j)**," be substituted.

(6) That the directions for preparing Kraut's reagent, *Ibid.*, **(m)**, be deleted and the expression, "Prepare as directed under **176 (b)**," be substituted.

REPORT ON HYPOPHOSPHITES

By Henry R. Bond (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The quantitative determination of hypophosphites in sirup preparations by bromine oxidation was subjected to collaborative study this year, the slightly modified method of assay devised by C. F. Bruening[1] being used.

Because of the good results obtained last year in brief experimental assays, it was decided to use as material for collaborative work three sirups prescribed in the National Formulary VI, (1) Sirup Ammonium Hypophosphite, (2) Sirup Hypophosphites, and (3) Compound Sirup of Hypophosphites. A quantity of each was prepared from hypophosphites previously assayed by the methods outlined in the National Formulary VI for the simple salts.

A sample of each sirup was sent to each collaborator. The method submitted was published in *This Journal*, **22**, 90.

The results obtained by the collaborators are shown in the table.

DISCUSSION

The results of collaborative study are in good agreement with the calculated theoretical, which was obtained from assay results by National Formulary VI methods with the individual hypophosphite constituents. Such agreement among results is a strong indication that the non-hypophosphite ingredients (sugar, glycerol, etc.) have little or no effect on the bromine reagent when present in the concentrations prescribed by the National Formulary.

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[1] *J. Am. Pharm. Assoc.*, **25**, 19 (1936).

(a) *Effect of other ingredients on bromine reagent.*—For purpose of verification, determinations were made by the Associate Referee, who used

Collaborative results

COLLABORATORS* SIRUPS	AMMONIUM HYPOPHOSPHITE G. H_3PO_2/100 CC.	HYPOPHOSPHITES G. H_3PO_2/100 CC.	COMPOUND HYPOPHOSPHITES G. H_3PO_2/100 CC.
W. F. Reindollar, Baltimore	2.80	5.01	5.40
	2.80	4.98	5.42
	2.82	5.04	5.47
I. S. Shupe, Kansas City	2.85	5.05	5.51
	2.85	5.05	5.51
Jonas Carol, Cincinnati	2.76	4.91	5.39
	2.76	4.91	5.41
	2.76	4.92	5.41
C. B. Stone, Cincinnati	2.77	4.93	5.42
	2.77	4.93	5.42
H. G. Underwood, Cincinnati	2.77	4.94	5.41
	2.77	4.94	5.42
M. L. Yakowitz, San Francisco	2.84	5.04	5.52
	2.83	5.06	5.52
N. E. Freeman, Atlanta	2.82	5.05	5.52
	2.94	5.08	5.58
C. A. Wood, New York	2.82	4.98	5.44
	2.82	4.98	5.44
		4.99	5.45
H. R. Bond, Chicago	2.80	5.00	5.39
	2.80	5.01	5.39
	2.81	5.01	5.40
Average	2.81	4.99	5.45
Calculated theoretical	2.79	5.01	5.41
Average per cent of theoretical	100.71	99.60	100.74

* W. F. Reindollar, Maryland State Dept. of Health; all others, U. S. Food and Drug Adm.

preparations composed of the proper proportions of all ingredients except the hypophosphites; also, the individual non-hypophosphite ingredients were assayed in solutions of the same concentration of ingredients as in the prepared sirups. Average results of assays follow:

SIRUP PREPARED AS PRESCRIBED BY N. F. VI BUT WITH HYPOPHOSPHITES OMITTED	EFFECT ON REAGENT (CALCULATED AS G. H_3PO_2/100 CC.)
Ammonium hypophosphite	0.042
Hypophosphites	0.042
Compound hypophosphites	0.071
Individual non-hypophosphite ingredients	
Sucrose	0.042
Glycerol	0.000
Sodium citrate	0.000
Quinine and strychnine	0.034

The results of these determinations indicate that the non-hypophosphite ingredients exercise a reducing action upon the bromine reagent productive of slightly higher assay results. If corrections are applied to the average of the collaborators' results for the reducing action of these ingredients, the percentage relationship between the corrected average results and the calculated theoretical hypophosphite content of the sirups assayed is—

	per cent
Ammonium hypophosphite	99.28
Hypophosphites	98.80
Compound hypophosphites	99.46

However, since each aliquot used in the assays is equivalent to only 1 cc. of the original sirups, the effect of the other ingredients on the bromine reagent is so small that it falls within the limits of experimental accuracy among collaborators.

(b) *Stability of sirups.*—The stability of the hypophosphite content of the prepared sirups is illustrated by the fact that results of assays made seven months after compounding were as close to the theoretical figure as were results obtained from assays of the freshly prepared sirups, an indication that no oxidation of the hypophosphites had occurred.

(c) *Scope of applicability of the method.*—The bromine oxidation method devised by Bruening may be used in the assay of the hypophosphite sirups designated as official by the National Formulary, and also in the assay of such non-official sirups as contain no reducing agent other than hypophosphites, and no material of a phenolic character with which the bromine might react in the manner of Koppeschaar's reagent.

(d) *Bromide-bromate reagent.*—Supplementary assays were made by the Associate Referee, who substituted for the prescribed 0.1 *N* bromine solution, the standard bromide-bromate solution prepared as directed in *Methods of Analysis, A.O.A.C.*, 1935, p. 551. On the basis of assay results, the A.O.A.C. solution (3 grams $KBrO_3$ and 12 grams of KBr per liter) may be substituted for the prescribed solution.

RECOMMENDATION[1]

It is recommended that the volumetric bromine oxidation method of Bruening, with the A.O.A.C. standard bromide-bromate solution, be adopted as tentative for the quantitative determination of total hypophosphites in official National Formulary sirup preparations and in non-official sirups in which the non-hypophosphite ingredients are qualitatively and quantitatively similar to the official sirups, and that the subject be closed.

REPORT ON DAPHNIA METHODS

By ARNO VIEHOEVER (Philadelphia College of Pharmacy and Science, Philadelphia, Pa.), *Associate Referee*

The use of daphnia as a biological reagent or testing tool has been considerably extended. Its use for the evaluation of cathartics has been recommended in previous reports of the Associate Referee on Rhubarb and Rhaponticum, published in *This Journal,* and it was also suggested in a paper published in another journal.[2] William Tinsley,[3] of the Laboratory of Pharmacology and Therapeutics, College of Medicine, Chicago, Ill., has used the daphnia successfully in the study of cathartic action. He adopted the Vie-tubes for observation and also designed a perfusion stage for observations on daphnia.[4]

N. Tischler and the writer[5] have used daphnia in the testing of insecticides (especially naphthylisothiocyanate), and H. Mack and the writer[6] have used it for the testing of toxic as well as laxative substances isolated from May apple root, *Podophyllum peltatum.*

Various other workers and laboratory organizations have become interested in experimenting with daphnia, e.g., Dr. Ned Proescher of Santa Clara County Hospital, San Jose, Calif.; Mr. Malin, Chemist of the Immunity Research Laboratories, Glendale, Calif.; Mr. Raybin of the Chemical Laboratory, New York City Health Department; and Dr. Fanto of the McKesson and Robbins Research Laboratories.

Testing of snake venoms and antivenins with daphnia has been carried out in the referee's laboratory with the assistance of Mr. Quimba, of digitalis standardization with Messrs. Sokoloff and Taransky; and research on other problems, indicated below, with Dr. Isadore Cohen.

The following assays are suggested for tentative methods:

APPROXIMATE ASSAYS FOR CANNABIS (MARIHUANA)[7]

Make a simple test for the narcotic principle in cannabis by extracting a small representative sample (e.g., 10 grams) for 30 seconds with a fairly stable reagent

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[1] For report of Subcommittee B and action by the Association, see *This Journal,* 22 56, 90 (1939).
[2] *J. Am. Pharm. Assoc.,* 27, 668 (1938).
[3] *J. Lab. Clin. Medicine,* 23, 985–990 (1938).
[4] *Ibid.,* 1076.
[5] *Soap,* 14, 109–123 (1938).
[6] *J. Am. Pharm. Assoc.,* 27, 632 (1938).
[7] Arno Viehoever, *Am. J. Pharm.,* 109, 1 (1937)

(3 cm.) consisting of isopropyl alcohol, containing .05 per cent sodium hydroxide, and .05 gram of highly adsorbent charcoal (e.g., Nuchar 000) added with the cannabis.

A positive test, recognized by the practically immediate appearance, after filtering, of a color change from almost colorless to pinkish to deep red evidently indicates the presence and amount of cannabinol. The approximate amount apparently can be determined colorimetrically.

Spontaneous evaporation of this reddish liquid leaves a dry, partially pinkish to violet and rather persistent residue. This dissolves to an orange red solution in strong ammonia and with a violet to almost bluish violet color in acetone.

Biological Method.—Triturate the benzene-free residue of a benzene solution of 0.1 gram in about 2 cc. of culture water, containing possibly 0.1 per cent of acetone to speed solution of cannabinol.

The narcotic effect of cannabis extract becomes especially noticeable by comparing the swimming behavior of the daphnia in the culture water containing cannabis, with daphnia swimming in the cannabis-free culture medium, without or with acetone.

So-called locomographs, recorded in the comparoscope, indicate the lowered level of swimming, the reduction in speed and distance of swimming, as measured by the same time unit chosen under the influence of narcosis. So-called "shadowgraphs," obtained by the simultaneous photographing of daphia in 2 chambers, one with, the other without cannabis, permit the observation of the lowered level of swimming in the case of cannabis, causing a progressive debility, leading to death. The progressive speed and extent of narcotic debility evidently depend upon the concentration.

APPROXIMATE BIOLOGICAL ASSAY FOR VITAMIN E AND TOCOPHEROLS[1]

I. *Preparation of vitamin E. deficient media.*—Exhaust Wizard sheep manure (20-mesh powder) with petroleum ether. Use 1 gram of this extracted manure to 1 gallon of dechlorinated tap water for the preparation of the deficient culture medium.

II. *Transfer of daphnia magna.*—Transfer gravid females into this medium from a normal Wizard sheep manure culture. A medicine dropper with the tip cut off has served well for this purpose, as practically no liquid need be transferred with it, if the analyst traps the daphnia within the dropper, removes liquid inside and out with blotting filter paper, and inserts the dropper for the release of daphnia into the deficient medium.

III. *Breeding of vitamin E deficient daphnia.*—Provide the same cultural conditions as for normal standardized daphnia. Separate any young that might be born between the 10th and 20th day. From this deficient culture medium select 20-day-old daphnia and record ovarian development as follows: − none; + oocytes visible; ++ bluish green colorative in ovaries, individual eggs roughly outlined, gravid; with number of embryos released in brood sac.

IV. *Restoration of deficiency.*—Place groups of 10 daphnia in separate museum jars, 4.8×10×15 cm., filled with 8 ounces of the same deficient culture medium, using one jar for control and at least two jars for the experiments with the test substance in amounts of 1 mg., or in quantities below or above that amount. Follow the general growth and the internal development carefully. Note and record especially whether the ovarian dysfunction is removed and the rapid reproductive cycle or rhythm is reestablished, grading the ovarian response as before.

V. *Comparative tests.*—For comparison observe the effect upon daphnia placed in culture medium with four drops of reasonably fresh wheatgerm oil (Tritricol)

[1] Viehoever and Cohen, *Am. J. Pharm.*, **110**, 1 (1938).

added to a gallon of this vitamin E deficient medium,—resulting in the cure of ovarian dysfunction and the reestablishment of the reproductive rhythm.

VI. *Increased fecundity.*—Grow daphnia in groups of 10 in museum jars filled with 8 ounces of regular Wizard or Bovung medium under uniform conditions and determine the distribution curve for the number of young released in the initial clutches. Add the substance (mg. quantities) to groups of 1 day old daphnia, grow under like conditions, and determine their distribution curve for the number of young in the clutches. A significant increase strongly indicates vitamine E properties of the test substance.

BIOLOGICAL ASSAY FOR APHRODISIACS AND IRRITANTS[1]

I. *Breeding of male daphnia magna.*—Maintain crowded cultures of daphnia at sub-optimum nutritional conditions. Select mature vigorous males.

II. *Standard reference substances.*—Cantharidin in solution of culture water (1.0 gram: 30,000 cc.); or yohimbine in solution of culture water (0.1 gram: 100 cc.).

III. *Preparation of daphnia for the test.*—Evacuate the intestine with 1:100 dilution of alcohol-free fluidextract of cascara sagrada. Return daphnia to culture medium for 1 hour, then mount the daphnia in hanging drop preparations of solutions of cantharidin, of yohimbine, and of the test substance at known and comparable concentrations.

IV. *Observations made with compound microscope.*—

(1) Note effect on penis-like male sex organ—of both daphnia without and with evacuated intestine, observing increased movement of the excited sex organ and possibly the ejaculation of sperm.

(a) Compare reaction carefully with cantharidin, if ejaculation occurs.

(b) Compare with yohimbine, if only increased movement of the excited sex organ occurs.

(2) Record local effects upon the tissues and especially the mucosa of intestine, and general effects upon various organs and organ functions as intestine, liver, kidney, heart beat, respiratory rate, and eye movement.

(3) If tests show similarities (a) to cantharidin action, compare as in general toxicity procedures with cantharidin 1:55,000, as standard reference solution; (b) to yohimbine action, compare similarly with .1% yohimbine hydrochloride as the standard reference solution.

BIOLOGICAL ASSAY OF TOXIC SUBSTANCES[2]

I. *Standardization of daphnia.*—Maintain and standardize the cultures as reported by Viehoever, and Viehoever and Cohen. The use of daphnia grown in the same medium (at 68°–72°F. in northern light) is recommended for a series of comparative tests.

II. *Conditions of environment.*—Preferably conduct the tests under the same light, temperature, etc.

III. *Standard procedure.*—Fit museum jars, measuring $14\frac{1}{2} \times 9\frac{1}{2} \times 1\frac{1}{2}$ cm. with 100 cc. of the test solution, made with the test solution used for the growing of test daphnia. Divide the jars by markings or lines on the outside into as many equal zones as are necessary for the preciseness of the test (a detail adjusted for the particular toxic agent in question). Use 50 daphnia, as the minimum, for every test.

IV. *Theory of test.*—The normal functioning of the swimming mechanism of the daphnia is so characteristic that any impairment to it can be considered the result of a toxic influence. Four general reactions are produced, depending on the nature

[1] Viehoever and Cohen, *Am. J. Pharm.*, **110**, 6 (1938).
[2] *Ibid.*, **109**, 285 (1937).

of the agent: (1) Incoordination (impairment of normal swimming); (2) excitation, or even convulsions forcing the ascent of daphnia, followed by depression or paralysis, causing their descent; (3) progressive depression or paralysis; (4) precipitate depression forcing the more or less rapid descent.

All conditions being standard or constant, and the only variable being concentration, the following relationship holds true:

Debility of daphnia (inability to ascend) = concentration of test substance × time. The inability to ascend may be measured by arbitrary zones of locomotion. Count the number in zones at definite time intervals, and continue long enough to reach the end point at which all daphnia are down but not necessarily dead.

V. *Comparative tests.*—Examine the action of the standard reference agent upon the swimming mechanism of daphnia. Ten animals may be used for this purpose. If the reaction falls under IV, 1–3, proceed as follows: Establish the debility response for representative concentrations for 0.1, .01, .05, and .001%. The concentrations selected will be governed by the degree of toxicity of the agent. More dilute solutions tend to give more accurate results, although increasing the length of time for the necessary sequence of observations.

Check the test agents at concentrations where the standard reference causes a debility shift from 6–8 hours. Avoid higher concentrations since small differences in activity might not be readily observed. Compare carefully the swimming behavior in standard and test solutions.

VI. *Testing of single-active principles.*—Making additional observations where necessary, record the heart beat and respiratory rates of a representative number of daphnia at the start of the assay. Record the same at the end of the assay in standard reference and test solutions. This method of assay is suitable for single active principles, like strychnine, pilocarpine, picrotoxin, etc.

VII. (1) *Testing of U.S.P. tinctures and fluidextracts.*—If the products owe their activity to one important single substance, select this substance for use as the reference standard. Determine its action as suggested above. Determine the *p*H of the tincture or extract. If alkaline, add enough dilute tartaric acid to make it acid to litmus paper. Evaporate by fan at room temperature to highly viscous consistency. Dilute with distilled water to 5 times the original volume. Stir thoroughly and set aside for 15 minutes. Stir again and filter. Take 5 cc. of the filtrate as the equivalent of 1 cc. of tincture or fluidextract, whereas 5 cc. of filtrate + 95 cc. of culture water would equal 1–100 dilution of tincture or fluidextract. Proceed as indicated previously with a measured portion, using the same amount of the unfiltered mixture for a check.

(2) *Testing U.S.P. tinctures and fluidextracts.*—If they owe their activity to a chemical complex, select one lot as a reference standard and make relative comparisons, proceeding as in VIII. Grade on basis of fastest debility shift at the same concentration and in the same time.

VIII. *Testing of tinctures and fluidextracts* (*not U.S.P.*).—If they owe their activity to a chemical complex, prepare from a representative identified material a tincture or extract, according to U.S.P. method. Use this as a standard reference and proceed as previously directed.

Similar methods, with or without modification, may be used for testing such substances as veratrum[1] and marihuana, and for the evaluation of organic synthetics and their derivatives (e.g., amphetamine[2] etc.). Permanent records may be made with locomographs and shadowgraphs.

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[1] *Am. J. Pharm.*, 111, 3 (1939).
[2] *Ibid.*, 110, 12 (1938).

REPORT ON HEXYLRESORCINOL

By MORRIS L. YAKOWITZ (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

At the last meeting of the Association, a method for determining hexylresorcinol in olive oil was presented. This method was used by the associate referee and two collaborators in assaying a solution of hexylresorcinol in olive oil, which was made up to contain 1.486 per cent by weight of hexylresorcinol. The results obtained by the analysis indicated that the method was satisfactory.

After this known mixture had aged for about one year, it was assayed by F. A. Rotondaro of the Philadelphia Station. He obtained a recovery of 1.43 per cent by weight of hexylresorcinol, which is equivalent to 96.3 per cent of the amount added. He also carried a weighed amount of hexylresorcinol without olive oil through the method and obtained a recovery of 97.6 per cent. He then made a solution of hexylresorcinol in olive oil that contained 2.84 per cent by weight of hexylresorcinol. Assay of this solution gave a recovery of 2.76 per cent and 2.80 per cent, corresponding to 97.2 per cent and 98.5 per cent of the added hexylresorcinol.

Rotondaro's work indicated that the use of hydrazine in the method may be unnecessary. It is therefore recommended[1] that the method be studied during the coming year with the objective of simplifying it.

REPORT ON ERGOT

By LLOYD C. MILLER (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The colorimetric method, which was used in last year's collaborative work, was studied further. This method has been used to determine the amount of alkaloids extracted from crude ergot by ether (Soxhlet) and by the U.S.P. menstruum (Process C percolation with acidified 50 per cent alcohol). The latter extraction process appears to remove 50–75 per cent of the amount of alkaloid that can be extracted with ether.

REPORT ON NITROGLYCERIN IN MIXTURES

By OMER C. KENWORTHY (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

The method used last year appeared to be satisfactory, but when submitted to collaborators, unexplainably the results showed shortages in the neighborhood of 30 per cent.

This year an attempt was made to find out the reason for the shortages.

[1]For report of Subcommittee B and action by the Association, see *This Journal*, 22, 56 (1939).

Determinations were made by essentially the same method as last year, but certain procedures were modified. For example, the amount of acid was varied from 1 drop to 5 cc., and the time of shaking was varied from 5 minutes to 1 hour. Absolute alcohol instead of the ordinary 95 per cent alcohol was tried, and the acid was added both before and after filtering. All variations yielded a return of approximately 80–90 per cent of nitroglycerin.

When the amount of alcohol was increased to 100 cc. (50 cc. used previously) slightly higher results were obtained, but still they were approximately 10 per cent short. Attempts to extract the nitroglycerin with ether or amyl alcohol resulted in rather complete failures; an attempt to use alcohol neutralized to phenolphthalein gave a shortage of approximately 40 per cent.

A series of determinations was made with 50 cc. of alcohol. The liquid was centrifuged and filtered into a 200 cc. volumetric flask, and the process was repeated with 25 cc. of alcohol, until 200 cc. of filtrate was obtained. Nitroglycerin was run on a 100 cc. aliquot, by the method outlined last year, except that no acid was present in the alcohol, 2 cc. being added just before distilling. Six such determinations gave an average recovery of 95 per cent. An attempt to control the acidity of the powdered extracts by using quinine alkaloid resulted in a recovery of about 75 per cent.

Since a recovery of 95 per cent could be obtained by centrifuging and making to volume, this method, with a new supply of nitroglycerin, was submitted to collaborators. Due to the nearness of the annual meeting it was submitted before the associate referee had completed his work. The results of three analysts from the Food and Drug Administration who used the method follow:

	grain/tablet	
J. C. Molitor, New York City	0.0203	0.0206
E. H. Grant, Boston	0.0199	0.0194
O. C. Kenworthy	0.0195	0.0202

As the tablets contained 0.0236 grain/tablet (av. of 6 determinations) it can be seen that the shortages on the collaborative samples amount to 15–20 per cent.

Grant commented as follows: "There was still much green color in the last 25 cc. portion of alcohol, but theoretically there should be about 99.5 per cent extraction of the nitroglycerin."

This year as last year, a method that seemed promising failed to give good results when tried by collaborators. No method so far tried has resulted consistently in a good recovery of nitroglycerin. It is recommended[1] that the work be dropped temporarily.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 56 (1939).

REPORT ON GUAIACOL

By KENNETH L. MILSTEAD (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The Viebock and Schwappach method as modified by E. P. Clark for the determination of alkoxyl groups was applied to the determination of guaiacol and guaiacol derivatives last year. This year the method was subjected to collaborative investigation. Two samples were sent to collaborators.

Sample No. 1 consisted of guaiacol carbonate and conformed to N.F. VI purity tests.

Sample No. 2 consisted of synthetic guaiacol.

The method was published in *This Journal*, **22**, 91, 100.

Owing to the special apparatus required for this determination only a limited number of collaborators are available. Results were obtained from four on guaiacol carbonate and from two on guaiacol. The findings of the collaborators and the results obtained by the Associate Referee follow.

Collaborative Results

COLLABORATOR	SAMPLE NO. 1 GUAIACOL CARBONATE FOUND	SAMPLE NO. 2 GUAIACOL FOUND
M. Harris	99.8	
Chicago	99.9	
	99.8	
S. M. Stark, Jr.	100.0	
St. Louis	99.7	
E. P. Clark	98.7	98.4
Washington	98.2	98.6
S. Reznek	100.5	99.3
New York		100.0
Associate Referee	99.8	98.6
	99.9	98.3
	99.8	

The determinations by Clark were made on samples weighing between 13 and 15 mg., and 0.05 *N* thiosulfate was used.

Comment of Collaborator S. Reznek.—I took the liberty of making the following modifications, which I have used in routine work with the apparatus:

(1) Use of 3 cc. of liquefied phenol (90% phenol—10% water), instead of 2.5 cc. of crystalline phenol, to avoid the necessity of melting the phenol.

(2) Use of a single 15 ml. test tube containing 10–12 cc. of bromine solution, instead of the two receivers "C" and "D."

It is recommended:[1] That the Viebock and Schwappach method as modified by E. P. Clark for the determination of alkoxyl groups be adopted as a tentative method for the determination of guaiacol and guaiacol carbonate.

No report on biological testing was given by the associate referee.

REPORT ON IODINE OINTMENT

By William F. Reindollar (State of Maryland Department of Health, Baltimore, Md.), *Associate Referee*

Iodine ointment is a suspension of a glycerol solution of iodine and potassium iodide in a base composed principally of petrolatum. When

Collaborative results on iodine ointment

COLLABORATOR	SAMPLE A				SAMPLE B			
	FREE IODINE		ORGANICALLY COMBINED IODINE		FREE IODINE		ORGANICALLY COMBINED IODINE	
		AV.		AV.		AV.		AV.
H. R. Bond	3.27		0.98		3.66		0.07	
	3.25	3.26	0.93	0.95	3.64	3.65	0.07	0.07
W. D. Dembeck			0.70				0.06	
			0.78				0.06	0.06
			0.82					
			0.85	0.79				
H. E. Chaney	3.32		0.33		3.65		0.08	
	3.29	3.31	0.45	0.39	3.64	3.65	0.07	0.08
J. C. Jones	3.24		0.66		3.47		0.07	
	3.22		0.72	0.69	3.51		0.06	0.07
	3.47				3.51			
	3.46	3.35			3.49	3.50		
H. J. Fisher	3.35		0.39		3.64		0.11	
	3.40	3.38	0.40		3.67	3.66	0.12	0.12
			0.48	0.42				
J. W. Todd	3.29	3.29	0.17	0.17	3.50	3.50	0.17	0.17
L. T. Ryan	3.40		0.56		3.53		0.10	
	3.43	3.42	0.54	0.55	3.51	3.52	0.10	0.10
Referee	3.29		0.38		3.61		0.07	
	3.32	3.31	0.34	0.36	3.55	3.58	0.07	0.07

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 56 (1939).

examined under a magnification of 80 diameters these glycerol droplets may be plainly seen dispersed throughout the base. Sampling errors are very likely to occur with such a heterogeneous compound, particularly after exposure to extremes of temperature. To minimize this difficulty each batch of ointment was mixed well and stored in a 20° incubator.

The present tentative method for iodine and the proposed method for organically combined iodine were subjected to collaborative study. Two samples, one an ointment four years old labeled A, the other a freshly prepared ointment labeled B, were sent out with the request that they be mixed well before being analyzed. The results appear in the table.

Iodine.—No difficulty was reported by any of the collaborators with this procedure. Duplicate determinations of each worker show good agreement, and the small variation occurring among the several sets of results may be attributed to sampling errors or to time intervals occurring between analyses.

Organically Combined Iodine.—Although the results of a majority of the collaborators showed good agreement on the fresh sample, this was not the case with the older one. It was found very difficult to extract all the iodide from the base, in some instances more than thirty washings being required. It is apparent, therefore, that this procedure, while perhaps satisfactory for freshly prepared ointments, is unsuited for general work.

RECOMMENDATIONS[1]

It is recommended—

(1) That the present tentative method for the determination of iodine in iodine ointment be adopted as official, first action.

(2) That the study of methods for the determination of organically combined iodine in iodine ointment be discontinued.

REPORT ON THE SEPARATION OF ACETYLSALICYLIC ACID, ACETPHENETIDIN, AND CAFFEINE

By DONALD C. GROVE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The problem of effecting a quantitative separation of acetylsalicylic acid, acetphenetidin, and caffeine in admixture has been studied by several investigators.

Methods of Analysis, A.O.A.C., 1935, 553, contains an official method for the determination of acetylsalicylic acid in mixtures containing acetphenetidin and caffeine. This method was developed by Harrison, *This Journal*, **8**, 499. It, however, is a method for determining only the acetylsalicylic acid, the acetphenetidin and caffeine both being discarded.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 57 (1939).

Hitchens[1] presented experimental data showing that acetylsalicylic acid could be separated satisfactorily from a number of medicinals, including acetphenetidin and caffeine by means of sodium bicarbonate. The acetylsalicylic acid could then be recovered by acidification of the bicarbonate solution and extraction with a suitable solvent.

Berman, *This Journal,* **19,** 520 also effected a successful separation of the acetysalicylic acid from the acetphentidin and caffeine by means of sodium bicarbonate. For the separation of acetphenetidin and caffeine he tried several different methods, which did not prove very satisfactory in the hands of his collaborators.

The method as worked out in the present investigation employs the separation of acetylsalicylic acid from acetphenetidin and caffeine by means of sodium bicarbonate. The acetphenetidin and caffeine are then treated with a dilute solution of sulfuric acid, which removes all the caffeine plus a small quantity, usually about 75 mg., of acetphenetidin, leaving the main bulk of the acetphenetidin behind. The caffeine is then separated from the remaining acetphenetidin by means of the acid hydrolysis method of Emery, *This Journal,* **2,** 63, which is a tentative method, *Methods of Analysis, A.O.A.C.,* 1935, 548.

Of the three ingredients to be determined, caffeine is most difficult to recover quantitatively. This is apparent for several reasons. First, the caffeine is present in the smallest proportion; second, it is the last ingredient determined and thus contains any errors of manipulative technic from the separation of the other two ingredients; third, if the hydrolysis of the acetphenetidin is not complete, any unconverted acetphenetidin will be weighed with it; and finally, extracted tablet lubricants may be weighed with the caffeine.

It is because of these conditions that a preliminary separation of the caffeine from as much of the phenacetin and tablet lubricants as possible, by means of weak sulfuric acid, was decided upon. The method also has the advantage of allowing a larger sample to be taken for assay and the small amount of phenacetin is hydrolyzed more completely.

The method that was sent out to collaborators was published in *This Journal,* **22,** 91 (1939).

COLLABORATIVE WORK

Two samples were sent out for collaborative work. Sample No. 1 was a mixture prepared by the Associate Referee to contain acetylsalicyclic acid, 56 per cent; acetphenetidin, 40 per cent; and anhydrous caffeine, 4 per cent.

Sample No. 2 was a commercial compressed tablet stated by the manufacturer to contain, per tablet, acetylsalicylic acid, 0.1764 gram; acetphenetidin, 0.1176 gram; and caffeine, 0.0294 gram.

[1] *J. Am. Pharm. Assoc.,* **24,** 1084 (1934).

TABLE 1.—*Collaborative results*

Sample No. 1.—Per cent

COLLABORATOR	ACETYLSALICYLIC ACID			ACETPHENETIDIN			CAFFEINE ANHYDROUS		
	FOUND	THEORETICAL	AV. RECOVERY	FOUND	THEORETICAL	AV. RECOVERY	FOUND	THEORETICAL	AV. RECOVERY
C. F. Bruening Food & Drug Adm., Baltimore	54.58 54.65	56.00	97.5	41.23 41.01	40.00	102.8	4.14 4.10	4.00	103.0
H. H. Shull McNeil Lab., Philadelphia	54.92 54.97	56.00	98.1	41.40 40.99	40.00	103.0	4.24 4.31	4.00	106.9
L. E. Warren Food & Drug Adm., Washington	54.38 54.18	56.00	96.9	41.50 41.29	40.00	103.5	4.03 4.02	4.00	100.6
W. J. Watkins The Upjohn Co., Kalamazoo	54.90 54.86	56.00	98.0	39.65 39.99	40.00	99.6	3.88 3.97	4.00	98.1
D. C. Grove	55.64 55.71	56.00	99.4	40.44 40.36	40.00	101.0	4.16 4.14	4.00	103.8
Average	54.88	56.00	98.0	40.79	40.00	102.0	4.10	4.00	102.5

Sample No. 2.—Gram/tablet

	FOUND	DECLARED	AV. RECOVERY	FOUND	DECLARED	AV. RECOVERY	CAFFEINE + H_2O		
							FOUND	DECLARED	AV. RECOVERY
			per cent			*per cent*			*per cent*
C. F. Bruening	0.1785 0.1778	0.1764	101.0	0.1166 0.1174	0.1176	99.5	0.0301 0.0302	0.0294	102.6
H. H. Shull	0.1762 0.1764	0.1764	99.9	0.1166 0.1177	0.1176	99.6	0.0304 0.0303	0.0294	103.2
L. E. Warren	0.1764 0.1761	0.1764	99.9	0.1178 0.1180	0.1176	100.3	0.0303 0.0309	0.0294	104.1
W. J. Watkins	0.1744 0.1738	0.1764	98.7	0.1111 0.1092	0.1176	93.7	0.0292 0.0273	0.0294	96.1
D. C. Grove	0.1717 0.1726	0.1764	97.6	0.1190 0.1184	0.1176	100.9	0.0304 0.0304	0.0294	103.4
Average	0.1754	0.1764	99.4	0.1162	0.1176	98.8	0.0300	0.0294	101.9

The results and comments of the collaborators follow:

COMMENTS OF COLLABORATORS

C. F. Bruening.—The method gave no manipulative difficulties, but considerable time was involved in evaporating the phenetidin sulfate solutions until all the acetic acid was removed.

H. H. Shull.—It takes considerable time to run the samples by this method, but the results indicate that the method is the best we have tried for this separation. The method for aspirin is satisfactory as it stands. Titration of the residue indicates purity of 99.5 per cent aspirin. Some operators think that caffeine should be dried at 80° C. to constant weight, instead of at 100° C., to prevent loss.

W. J. Watkins.—The method we have been using for years on such mixtures is similar to yours and, in our opinion, is reasonably satisfactory. It took considerable time to reach constant weight in drying the phenacetin after conversion from phenetidin, but aside from that your proposed method works well enough.

The agreement between collaborators is believed to be as satisfactory as could be expected in a mixture of this type, where all three ingredients are determined quantitatively on a single sample. Therefore it is recommended[1] that the proposed method for the determination of acetylsalicylic acid, acetphenetidin, and caffeine be adopted as tentative, and that the subject be closed.

REPORT ON GUMS IN DRUGS

By J. H. CANNON (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

During five years various methods for the identification of gums in drug products have been considered by the Associate Referee. Two years of collaborative study of tests depending upon the formation of characteristic precipitates from aqueous mixtures indicated that such a procedure is open to several objections, of which the most serious, perhaps, is that the concentration of gum present in an unknown is not readily ascertained, *This Journal*, **19**, 528. This, together with the fact that the quality of certain precipitates seems to vary with the proportion of gum present, suggested that tests applied to the precipitated gum itself might be more dependable than precipitation reactions. Accordingly, a study was made by the Associate Referee of published tests of this type, principally those described in the literature of pharmacognosy and plant histology, and generally depending upon staining or color formation with the usual reagents used in microscopy.

Collaborative study on identifications of this type was made this year. Six unknowns were submitted, as follows: I (Irish moss), II (tragacanth), III (agar), IV (quince), V (karaya), and VI (galagum). Control samples of these gums and instructions to collaborators were sent with the unknowns.

The method was published in *This Journal*, **22**, 92.

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[1]For report of Subcommittee B and action by the Association, see *This Journal*, **22**, 57, 91 (1939)

NOTE ON OUTLINE OF TESTS

Unlike the work on microchemical identification of alkaloids, the present scheme provides for confirmatory tests as well as elimination tests because, generally speaking, no single reaction is sufficient to identify any of the gums. In this connection the reactions of tragacanth may be cited as an illustration. With certain samples a rapid blue-black stain is produced with chlorzinc iodide. With other authentic samples the blue color appears more slowly. The microscopical characteristics of these two types are distinct. This possibly is due to differing methods of production of the crude gum, certain types being the knotty yellow or brown natural exudation, other types being the pure white or semi-transparent ribbons resulting from an incision in the bark of the shrub near the base. The latter is now said to be the favorite form, although the tragacanth of commerce is a conglomerate mixture of good, bad, and indifferent as obtained from the caravans.[1] There is also the possibility that various supposedly authentic samples of tragacanth may have been derived from different species of *Astragalus*, since, according to the literature, numerous species belonging to this genus yield a gummy matter having the properties of tragacanth.

Results of Collaborators

	I	II	III	IV	V	VI
F. J. McNall U. S. Food & Drug Adm. Chicago	Irish Moss	Tragacanth	Agar	Quince	Karaya	Galagum
E. C. Payne U. S. Food & Drug Adm. Chicago	Irish Moss	Tragacanth	Agar	Quince	Karaya	Galagum
J. Claggett Jones State Div. Chemistry Richmond	Irish Moss	Tragacanth	Acacia*	Quince	Karaya	Galagum
C. E. Shepard Conn. Agr. Exp. Sta. New Haven	Irish Moss	Tragacanth	Agar	Quince	Acacia*	Galagum
Waldo L. Scoville Mich. State Dept. Agr. Lansing	Irish Moss	Tragacanth	Agar	Quince	Acacia*	Galagum
J. Reilly U. S. Food & Drug Adm. New York	Irish Moss	Tragacanth	Agar	Quince	Karaya	Galagum

* These incorrect identifications were believed to be due to the use of too small a portion for testing. Accordingly, additional samples were submitted to these three collaborators, identified as VII, VIII, and IX. VII contained acacia, VIII contained karaya, and IX contained agar. All three collaborators identified these samples correctly.

[1] John Uri Lloyd, Origin and History of Pharmacopial Vegetable Drugs, 1921.

COMMENTS BY COLLABORATORS

McNall.—Samples I, II, IV, and V were easily identified. With Sample III, I experienced some difficulty in getting a suitable precipitate free of mineral oil. The original precipitate came down fine and flocculent and after washing twice with ether it still contained enough oil to interfere with the test with tincture of iodine. The precipitate was again washed with ether, dissolved in water, and reprecipitated with alcohol. A mat was obtained which gave a good color reaction with iodine. The pink coloration with concentrated sulfuric acid and galagum is rather difficult to obtain since it is very fleeting.

Payne.—Little trouble was experienced when unknowns were compared directly with known samples. The characteristic structures in Irish moss were not always seen. The test somewhat resembles that for tragacanth. The pink color produced by hydrochloric acid on karaya may be easily missed but is definite. I would suggest that the reaction of every gum with all four group reagents be described in order to aid in sorting out.

Shepard.—All of the gums were stained pink with ruthenium red but probably karaya was stained deepest pink of all.

Jones.—The results were none too satisfactory. This was probably due to my lack of experience in this method of identification.

Scoville.—The appearance of the iodine test seemed to vary considerably, due apparently to the thickness and dryness of the mat. Sample V gave fine precipitate but did not appear granular. It seemed to dissolve in ruthenium red rather than be colored by it. The sulfuric acid group test does not appear to be as satisfactory as is desirable.

Reilly.—Reactions of the various gums with group I and group II reagents seem characteristic and should be useful for the identification of those gums. Tests depending on the use of ruthenium red reagent were found unsatisfactory, due perhaps to the condition of the reagent on hand.* The color change of galgagum with sulfuric acid is striking enough. Heating should be done cautiously. The behavior of acacia with this reagent seemed not so characteristic. The change to a greenish brown as described in the outline is not so readily perceptible, the color gradually darkening. When available, larger quantities of gum solution might be used to advantage.

SUMMARY AND RECOMMENDATIONS[1]

Collaborative results show that the method submitted is satisfactory for the six gums studied when these occur unmixed in drug preparations.

It must be noted that comparison with controls is essential, and that a certain degree of familiarity with the appearance of the different gum precipitates is an important part of the method.

It is the opinion of the Associate Referee that no further work on this subject is indicated at this time, accordingly it is recommended that the method be adopted as tentative and that the subject be closed.

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* This reagent decomposes slowly and it is believed that this accounts for the collaborator's statement on this point.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22 57 ,92 (1939).

REPORT ON THEOBROMINE IN THEOBROMINE CALCIUM TABLETS

By P. S. JORGENSEN (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

Study of this subject was continued this year in accordance with the recommendation of the referee. Samples of powdered theocalcin tablets, theocalcin powder, and theobromine alkaloid were sent to the collaborators with instructions to assay by the proposed acidimetric method and the present tentative iodometric method.

INSTRUCTIONS TO COLLABORATORS

Powder the sample in a mortar and dry at 110° C. Place 0.5 gram of the powdered tablets or 0.4 gram of the theocalcin powder, or 0.2 gram of theobromine alkaloid in a 300 cc. beaker and add 100 cc. of water. Warm moderately over a flame and add 15 cc. of approximately 0.1 *N* H_2SO_4. Heat to boiling to insure complete solution and to remove CO_2. Cool to room temperature under the tap. Add 1.5 cc. of phenol red indicator, render slightly alkaline with approximately 0.1 *N* NaOH, and titrate carefully to an acid reaction with 0.1 *N* H_2SO_4 (yellow color). To this solution add 25 cc. (an excess) of neutral 0.1 *N* $AgNO_3$ and titrate the liberated HNO_3 immediately with 0.05 *N* NaOH to a distinctly violet red color. Titrate cautiously drop by drop with constant stirring near the end point.

1 cc. of 0.05 *N* NaOH = 0.009 gram of $C_7H_8O_2N_4$.

The results obtained are given in the table.

COLLABORATOR	PER CENT THEOBROMINE IN THEOBROMINE ALKALOID		PER CENT THEOBROMINE IN THEOCALCIN POWDER		PER CENT THEOBROMINE IN THEOCALCIN TABLETS	
	ACIDIMETRIC METHOD	IODOMETRIC METHOD	ACIDIMETRIC METHOD	IODOMETRIC METHOD	ACIDIMETRIC METHOD	IODOMETRIC METHOD
M. L. Yakowitz	100.79	99.31	48.28	47.44	41.43	41.77
Food & Drug Adm.	100.28	98.66	48.69	47.48	41.53	41.06
San Francisco	100.96		48.30		41.49	
Harry Isacoff	99.87		48.69		41.29	
Mutual Pharmacal Co.	100.28		48.98		41.55	
Syracuse	99.94		48.92			
P. S. Jorgensen	99.94	103.18	49.16	50.67	41.81	44.55
	100.17	102.24	49.27	50.89	41.81	44.55

A study of the results shows that the iodometric method gives varying results, while the acidimetric method gives consistent results in the hands of different analysts. Furthermore, the acidimetric method is easily carried out in a few minutes while the iodometric method requires standing overnight. Considering then the better results obtained by the acidimetric method and the ease with which it may be performed, the Associ-

ate Referee feels justified in recommending[1] that this method replace the present tentative method.

REPORT ON CHLOROBUTANOL

By FRANK C. SINTON (U. S. Food and Drug Administration, U. S. Department of Agriculture, New York, N. Y.), *Associate Referee*

Last year a collaborative study was made on the determination of chlorobutanol, and also chlorobutanol in aqueous solution. Although the results on the chlorobutanol crystals were reasonably in line with expectation, the findings on the solution showed marked discrepancies from the theoretical percentage of chlorobutanol. During the current year samples of chlorobutanol and chlorobutanol in solution were again submitted for collaborative study. The method for chlorobutanol was essentially the same as last year's, but in the method for chlorobutanol in solution some of the details of the distillation were changed.

The chlorobutanol used in the preparation of the collaborative samples was purchased from a reputable manufacturer and found to comply with the U.S.P. tests for purity. It was desiccated for four weeks and then found to have a melting point of 96° C.

The samples of chlorobutanol crystals were submitted to the collaborators in weighing bottles sealed with paraffin in order to avoid absorption of moisture. The sample of chlorobutanol in solution was of the same composition as that submitted to collaborators last year.

The method was published in *This Journal*, **22**, 95 (1939).

The results are shown in the table.

COMMENTS BY COLLABORATORS

Berman.—No manipulative difficulties were encountered. All solutions filtered before precipitation was made.

Hoshall.—The proposed method is well described, simple, and accurate, and no difficulty was experienced. We have been determining chlorobutanol by this method except that the chloride is determined volumetrically by the Volhard method instead of gravimetrically as silver chloride. Although the volumetric method may be a little more difficult from the manipulative standpoint, it is much more rapid than the gravimetric procedure.

Jorgensen.—The method is simple, and in my opinion constitutes a very good method for determining this substance.

Moraw.—Results on the solution do not check as well as I expected. Possible reasons that suggest themselves include the following: Incomplete saponification due to too much dilution of saponification solution, and incomplete washing down of condenser, adapter, etc.

I used a 22 inch condenser, but the experience with this suggests that a 14 inch condenser would be better because it would require less alcohol and water to

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 57, 94 (1939).

thoroughly wash it down. Incidentally the use of small amounts of water as a follow-up wash after alcohol results in the water "rolling" through the condenser like oil, without wetting the condenser and without having its full washing effect. As one observes this he logically concludes he should use more water. I am skeptical as to whether the water wash is necessary at all.

	CHLOROBUTANOL	CHLOROBUTANOL IN SOLUTION (4.500 GRAMS/1000 CC.)
	per cent	
S. M. Berman	99.46	4.35
Buffalo	99.53	4.32
Edward M. Hoshall	99.75	4.452
Baltimore	99.47	4.456
Paul S. Jorgensen	99.40	4.270
San Francisco	99.03	4.356
	98.65	4.331
H. O. Moraw	99.2	4.30
Chicago	99.6	4.39
		4.28
		4.29
F. A. Rotondaro	100.36	4.565
Philadelphia	99.61	4.573
C. A. Wood		4.39
New York		4.42
F. C. Sinton	99.[illegible]	4.48
[illegible]	[illegible].6	4.44
	99.6	4.45
Minimum recovery	98.65	4.27 (94.9%)
Maximum recovery	100.36	4.573 (101.6)
Average recovery	99.5	4.395 (97.7%)

For the determination of the chloride, the gravimetric and volumetric methods should be optional. If directions are given for either method they should agree in all respects with official or recognized procedures. For example, while it is doubtful if a difference of 5° in the drying temperature for the AgCl would make any difference in the results, since the U.S.P. directs drying it at 110° C. and Hillebrand and Lundell, 100° at first, then 130–150° C., it seems advisable to have your directions agree with one of these. Then the matter of acidity of the solution and precautions against too great excess of $AgNO_3$ and the washing solutions, temperature, standing, etc. should likewise be taken into consideration so as to conform more closely with recognized practice. Your directions for washing the AgCl differ from established methods.

The gravimetric method for AgCl is so widely published it should not be necessary to repeat it, but if reprinted it should be in agreement with the best authorities.

Rotondaro.—Since only an opalescence was obtained with the reagents used, no blank was deducted from the results.

No difficulty was encountered in the procedures outlined. Additional determinations were made in the case of the solution, 10 cc. of alcohol being added to the sample in addition to the 25 cc. of water. The resultant simultaneous distillation of chlorobutanol and alcohol kept the condenser and adapter clean. The washing of the condenser and adapter was then easily done with 5–10 cc. of alcohol and about 10 cc. of water. This modified procedure gave results of 99.68% in the chlorobutanol crystals and 0.4581 gram/100 cc. in the case of the solution.

CONCLUSION

Results obtained by the collaborators on the chlorobutanol crystals averaged 99.5 per cent, which constitutes a good recovery. In the case of the sample of chlorobutanol in solution the recoveries averaged close to 98 per cent of the theoretical, which appears to be quite reasonable for a determination of this nature.

The majority of the collaborators reported no difficulty with the method. In explanation of criticism by one of the collaborators regarding the method of determining the chloride it may be mentioned that for purposes of uniformity the method described in *Methods of Analysis, A.O.A.C.*, 1935, 579, for chloroform in mixtures was used for this purpose. No doubt any other official procedure, either volumetric or gravimetric, would be as accurate and acceptable since at this point the method involves simply the well known determination of chloride.

RECOMMENDATION[1]

It is recommended—

(1) That the method for the determination of chlorobutanol be adopted as tentative.

(2) That the method for the determination of chlorobutanol in solution be adopted as tentative.

(3) That the topic be discontinued.

REPORT ON PHENOLPHTHALEIN AND ACETYLSALICYLIC ACID

By George M. Johnson (U. S. Food and Drug Administration, Minneapolis, Minn.), *Associate Referee*

As a result of collaborative work done last year on this subject, the method was modified and a new mixture was prepared and sent out to collaborators for analysis. The mixture, made similarly to that used last year, contained 84.0 per cent of acetylsalicylic acid, 5.0 per cent of phenolphthalein, and approximately equal amounts of calcium carbonate, lactose, starch, and talc. The method used by the collaborators for the

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22 57 (1939

determination of phenolphthalein and acetylsalicylic acid in tablets modified as later suggested by the Associate Referee was published in *This Journal*, 22, 95.

The results obtained by the collaborators are given in the table.

Collaborative results

	ACETYLSALICYLIC ACID	RECOVERY	PHENOLPHTHALEIN (110°–120° C.)		RECOVERY AT 120°C.
	per cent	*per cent*	*per cent*		*per cent*
Henry R. Bond	75.07*			5.27*	
Food & Drug Adm.	80.27*			5.14*	
Chicago	79.65*			5.08*	
	76.27*			5.08*	
	74.93*			5.12*	
	83.33	99.2		4.83	96.6
	83.97	100.0		4.81	96.2
Jonas Carol	80.99	96.4	5.11	5.00	100.0
Food & Drug Adm.	81.03	96.5	5.20	5.12	102.4
Cincinnati	80.92	96.3	5.40	5.07	101.4
Irwin S. Shupe	82.7	98.5	5.52	5.12	102.4
Food & Drug Adm.	83.3	99.2	5.41	5.11	102.2
Kansas City					
McNeil Laboratories	84.1 H.H.S.	100.1	5.26	5.14	102.8
Philadelphia	83.2 H.H.S.	99.0	5.25	5.09	101.8
H. H. Schull	84.5 P.E.P.	100.6	5.41	5.16	103.2
Chief Chemist					
Llewellyn H. Welsh	82.83	98.6		5.03	100.6
Food & Drug Adm.	82.67	98.4		5.02	100.4
Baltimore	81.79*†				
	81.73*†				
C. A. Wood	82.9	98.7	5.22	5.16	103.2
Food & Drug Adm.	83.2	99.0	5.10	5.03	100.6
New York					
G. M. Johnson	83.64	99.6	5.14	5.06	101.2
	83.33	99.2	5.09	5.01	100.2
Average	82.91	98.7		5.05	101.0

* Not included in average.
† Samples accidentally allowed to evaporate to dryness on steam bath, although there was no acetous odor from decomposition.

The method is not specific on the details of the sodium bicarbonate extraction of the acetylsalicylic acid from the ether solution. For this reason two of the collaborators, Bond and Carol, obtained low results on the acetylsalicylic acid. Carol reports that he did not allow the bicar-

bonate solution to remain in contact with the ether solution except for a minimum time because of the possibility of hydrolysis of the acid. Bond conducted additional determinations, at the suggestion of the Associate Referee, allowing more time for the reaction between the acetylsalicylic acid and the bicarbonate to take place. These results on the acetylsalicylic acid are much closer to the actual content, although it is not known why low results were obtained on the phenolphthalein. If Carol's results on the acetylsalicylic acid are omitted, an average recovery of 99.2 per cent is shown, and with Bond's results on the phenolphthalein omitted, an average of 101.6 per cent is obtained, which results the Associate Referee considers a truer picture of the results possible by this method.

COMMENTS OF COLLABORATORS

Harry H. Schull.—The results check as closely as you would expect, considering the limitations of the gravimetric method. If the method is criticized because the end products weighed are not specific substances, it would be possible to determine the phenolphthalein as described on page 570, *A.O.A.C. Methods*, 1935, and the aspirin by one of the methods on page 551 of the same book.

Llewellyn H. Welsh.—The method entailed some manipulative difficulties because of the use of ether. In extraction of ether solution with 3 per cent alkali, there was a tendency toward seepage at the stopper and stopcock because of internal pressure.

A. C. Wood.—It is suggested that a titration might be used as a check on the purity of the aspirin residue. It also might be advisable to include a suitable warning in the method indicating that the bicarbonate extraction and isolation of the acid should be conducted as rapidly as possible in order to prevent hydrolysis.

DISCUSSION

The initial dry extraction of the sample is slow and tedious, but no solvent has been found that is an improvement on the ether. If sufficient time is allowed for neutralization of the acid by the sodium bicarbonate—each extraction being thoroughly shaken for about a minute—the solutions are kept cold, and the acetylsalicylic acid is extracted as soon as possible, practically quantitative results are obtainable.

Any acetylsalicylic acid not separated from the ether solution is eventually weighed as phenolphthalein, and it has been noticed that even with the most careful manipulation traces of salicylic acid are found in the phenolphthalein residue. It was for this reason that the Associate Referee suggested that the phenolphthalein residue be heated to constant weight at 120° C., rather than the customary 100° C., for at this temperature the traces of salicylic acid are volatilized.

It is recommended[1] that the method presented be adopted as tentative.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 57, (1939).

REPORT ON AMINOPYRINE AND PHENOBARBITAL IN MIXTURES

By E. C. PAYNE (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The method reported on last year, *This Journal*, **21**, 566–571, was tried by collaborators. The results, which are given in the table, were obtained with individually weighed samples of phenobarbital and aminopyrine. No excipients were added. For the preparation and purity of these substances see the same reference. The results follow:

ANALYST	EXCIPIENT	SUBSTANCE	TAKEN	RECOVERED	RECOVERY
			gram	*gram*	*per cent*
H. O. Moraw	None	Aminopyrine	0.3037	0.3023	99.5
H. O. Moraw	None	Phenobarbital	0.1031	0.1038	100.7
H. O. Moraw	None	Aminopyrine	0.4087	0.4071	99.7
H. O. Moraw	None	Phenobarbital	0.1545	0.1540	99.6
R. D. Stanley	None	Aminopyrine	0.3770	0.3743	99.3
R. D. Stanley	None	Phenobarbital	0.1046	0.1051	100.5

In addition, the following simple methods of separation were tried:

(1) *"Dry" extraction with CCl_4.*—Phenobarbital is slightly soluble in this solvent and it is therefore unsuitable.

(2) *"Dry" extraction with petroleum ether.*—While it appears that this solvent will eventually remove the aminopyrine, so many extractions are necessary that the process becomes unduly tedious. For example, twenty 30 cc. portions of solvent did not completely remove 0.4 gram of aminopyrine from 0.2 gram of phenobarbital.

(3) *Extraction of phenobarbital from acid solution ($1+9$ H_2SO_4) with chloroform or ether, and later removal of aminopyrine after making alkaline with ammonia.*—Samples of 0.1–0.2 gram of phenobarbital and 0.2–0.5 gram of aminopyrine were shaken with 25 cc. of the acid and the solution was extracted with the solvent until a test portion yielded no weighable residue. The solvent was washed with 5–10 cc. of water, which was added to the aqueous layer, filtered into a tared beaker and evaporated, and the residue was dried and weighed. The aqueous solution was made alkaline with ammonia and the aminopyrine extracted with chloroform, etc. The results are shown in the following table:

NO. OF SAMPLES	SOLVENT FOR PHENOBARBITAL	RECOVERY OF PHENOBARBITAL (AV.)	SOLVENT FOR AMINOPYRINE	RECOVERY OF AMINOPYRINE (AV.)
		per cent		*per cent*
8	Ether	102.4	Chloroform	96.6
3	Chloroform	102.6	Chloroform	98.5

The results indicate that separation is incomplete. Strangely enough, further extraction of the original acid solution with solvent yields no more extract. This might be explained by a slight interaction between aminopyrine and phenobarbital, a phenomenon known to occur at a somewhat higher temperature. Not enough results are yet available for definite conclusions to be drawn.

A somewhat different method was tried by L. E. Warren. He used the above method for aminopyrine, but extracted the phenobarbital in a continuous extractor, using chloroform as solvent. When he used individually weighed samples containing no excipients, his method gave 100.1–100.3 per cent recovery for aminopyrine and 100.4 per cent for phenobarbital. With similar samples containing lactose and starch he obtained 100.2 per cent recovery for aminopyrine and 101.5–102.3 per cent for phenobarbital.

In view of the small number of collaborative results and of the absence of excipients in the samples, the Associate Referee recommends[1] that the problem be reassigned to an associate referee for a study of the method when applied to tablets and other medicinal mixtures.

REPORT ON ELIXIR OF TERPIN HYDRATE AND CODEINE

By JONAS CAROL (U. S. Food and Drug Administration, Cincinnati, Ohio), *Associate Referee*

Last year a collaborative study of a method of analysis of elixir of terpin hydrate and codeine was made. Despite the small amount of codeine present in the elixir, very favorable recoveries were made by most of the collaborators. The results on terpin hydrate, however, were less satisfactory, being too high in all cases. Most of the collaborators considered that these high results were caused by condensation of moisture during the evaporation of the extract solution containing the terpin hydrate. This moisture apparently was not lost by subsequent drying of the residue without heat. The use of only a moderate amount of heat (50° C.) caused a constant loss of weight of the terpin hydrate residue. This was probably the result of loss of water of hydration in addition to water gained by condensation.

This year an attempt was made to find a satisfactory method of obtaining a dry terpin hydrate residue. In a preliminary study, aliquots of a solution of terpin hydrate in alcohol-chloroform solvent (7 per cent alcohol) were evaporated both by a current of air so as to condense moisture, and spontaneously. The residues obtained were then left exposed to air and weighed daily for one week. Results are shown in Table 1.

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[1] For report of Subcommittee B and action by the Association, see *This Journal* 22 57 (1939)

TABLE 1.—*Results on finding dry residue*

(Aliquots contained 0.1704 gram of terpin hydrate in 100 cc. 7% alcohol-chloroform mixture)

DAYS	SAMPLES EVAPORATED IN CURRENT OF AIR		SAMPLES ALLOWED TO EVAPORATE SPONTANEOUSLY	
0*	0.1731	0.1733	0.1715†	0.1711†
1	0.1731	0.1733	0.1715	0.1707
2	0.1729	0.1726	0.1702	0.1704
3	0.1724	0.1724	0.1709	0.1704
4	0.1728	0.1729	0.1705	0.1700
5	0.1726	0.1729	0.1703	0.1701
6	0.1725	0.1726	0.1703	0.1701
7	0.1725	0.1727	0.1702	0.1700

* Weighings began when residue was apparently dry.
† 35 hours from beginning of evaporation.
The weather during this week ranged from rainy and wet to hot and dry.

The results, Table 1, indicate (1) that contrary to the accepted view terpin hydrate loses water extremely slowly when exposed to air, and (2) that moisture gained by the residue during condensation is not lost again by exposure to air.

With the hope that spontaneous evaporation of the terpin hydrate extract was the solution to the problem, samples of elixir of terpin hydrate and codeine N.F. VI (containing 17 grams of terpin hydrate and 2 grams of codeine alkaloid per 1000 cc.) were sent to the collaborators to be analyzed by the following method:

METHOD

Terpin Hydrate.—Measure a 10 cc. sample from a small buret (allow to drain 5 minutes) into 10 cc. of water in a separator. Add 2 cc. of 10% H_2SO_4. Immediately extract (on standing crystals form and cause some inconvenience) with two 10 cc. portions of petroleum benzin and wash the combined petroleum benzin extracts with three 2 cc. portions of water to which 3–4 drops of dilute H_2SO_4 have been added. Petroleum benzin contains aromatics and may be discarded. Return acid washings to original separator and extract completely with alcohol-$CHCl_3$ solution (7% alcohol) to remove the terpin hydrate. (Seven extractions of 20 cc. portions should be sufficient.) Make an additional extraction and evaporate to dryness to test for complete extraction of terpin hydrate. Wash the combined alcohol-$CHCl_3$ extracts in a second separator containing 10 cc. of 2% H_2SO_4. (This wash is very important as glycerol carried over from original sample by $CHCl_3$-alcohol solution must be removed to prevent its being weighed with the terpin hydrate. The washing also prevents loss of codeine.)

Filter the alcohol-$CHCl_3$ extract through a pledget of cotton, previously wet with $CHCl_3$, into a tared crystallizing dish. Wash the dilute acid remaining in the separator with 10 cc. of the alcohol-$CHCl_3$ solvent, filter through cotton and add to the bulk of the solvent. Allow the combined solvent to evaporate spontaneously. (Experiments indicate that constant weight is reached in about 35 hours.) Report as terpin hydrate gram/100 cc.

Codeine.—Transfer the acid wash material to the original separator and make alkaline with ammonia. Determine codeine by the A.O.A.C. method 565, 69, be-

ginning line 5 ". . . extract 5 times with $CHCl_3$ . . . ." Report as gram/100 cc. of codeine. 1 cc. of 0.02 *N* H_2SO_4 = 0.00634 gram of $C_{18}H_{21}O_3N \cdot H_2O$.

Table 2 contains the results obtained by the collaborators and the Associate Referee.

TABLE 2.—*Collaborative results on terpin hydrate and codeine*

COLLABORATOR	TERPIN HYDRATE		CODEINE	
	G./100 CC.	% RECOVERY	G./100 CC.	% RECOVERY
C. B. Stone	1.701	100.1	0.199	99.5
Cincinnati	1.715	100.9	0.199	99.5
H. W. Conroy	1.750	102.9	0.203	101.5
Minneapolis	1.745	102.6	0.203	101.5
H. G. Underwood	1.719	101.1	0.198	99.0
Chicago	1.708	100.5	0.197	98.5
L. H. Welsh	1.742	102.5	0.200	100.0
Baltimore	1.735	102.1	0.198	99.0
	1.738*	102.2	0.198	92.0
	1.754*	103.2		
R. Hyatt	1.763	103.7	0.202	101.0
Cincinnati	1.767	103.9	0.200	100.0
Jonas Carol	1.697	99.8	0.201	100.5
Cincinnati	1.705	100.3	0.202	101.0
	1.709	100.5		
M. L. Yakowitz	1.790	105.3	0.180	90.0
San Francisco	1.768	104.0	0.187	93.5
I. S. Shupe	1.71	100.6	0.190	95.0
Kansas City	1.73	101.8	0.190	95.0
L. E. Warren	1.728	101.7		
Washington				

* Obtained by evaporating almost to dryness on the hot stone of a steam bath in a current of air, then removing dishes from steam bath and allowing them to stand in current of air for one hour.

The results again show that most of the analysts obtained very good recovery of codeine.

The results for terpin hydrate were appreciably better than the results reported last year. While Hyatt was making his determination the weather was very wet and rainy at Cincinnati, which might have caused his rather high result. Possibly moist weather at San Francisco also caused the high figure obtained by Yakowitz.

It is recommended[1] that the method be adopted as a tentative method.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 57 (1939).

REPORT ON EMULSIONS

By W. F. KUNKE (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Experimental work was continued as indicated in the report of last year and as recommended by Subcommittee B, *This Journal*, 21, 67. The limiting conditions of certain analytical details were determined and the proposed method was studied collaboratively. It is now recommended as a tentative method.

Last year it was shown that Soxhlet extraction with chloroform and with powdered pumice as a "spreader" gave incomplete extraction, 97.4 per cent recovery, even after a 9-hour period coupled with siphoning every 5 minutes, following standing overnight with chloroform in the extraction apparatus.

This year it was found that continued Soxhlet chloroform extraction ($8\frac{1}{2}$ hours) of the ingredients of the emulsion other than cod liver oil, namely, 0.5 gram of acacia, 1.5 cc. of water, and 0.4 cc. of sirup mixed with 20 grams of powdered pumice, yielded 0.0125 gram of residue after evaporation of the chloroform. This would introduce an error of +0.6 per cent on 2 grams of cod liver oil in a sample of an emulsion if the combined residue was not subsequently purified and assuming complete recovery of cod liver oil. Furthermore, when cod liver oil, 2.2773 grams, was dissolved in chloroform and the solution was refluxed in an acetylization flask on a steam bath for 10 hours, the cod liver oil, after evaporation of the chloroform, showed an increase in weight of 0.44 per cent. This was confirmed by another result of 0.45 per cent. Because of these unexpected results: (1) the material extracted when no oil was present, (2) the increase in weight of the oil due to a long period of refluxing, and (3) the low recovery, the Soxhlet extraction was abandoned.

Other methods of extraction of the cod liver oil from an emulsion were tried. (1) Extraction with chloroform of the emulsion previously mixed with a comparatively large volume of water was hopeless because of the very troublesome emulsion (strongly acidifying the mixture with hydrochloric acid did not improve matters). (2) The emulsion of cod liver oil was treated by mixing with 10 cc. of absolute alcohol, by stirring and evaporating the alcohol on a steam bath in the hope of "breaking" the emulsion in preparation for the extraction of the oil with chloroform (recovery was 90.6 per cent and 96.4 per cent). (3) The cod liver oil was extracted from the emulsion previously mixed in a beaker with a "spreader" to facilitate the extraction with chloroform (the most promising).

Various "spreaders" were tried, namely, powdered pumice, small pieces of filter paper, filter-cel, sodium chloride, magnesium oxide, crystalline calcium carbonate, and finely powdered calcium carbonate. Sodium chloride, magnesium oxide, and crystalline calcium carbonate were

unsatisfactory. Typical results for recovery of the cod liver oil from an emulsion by extraction with chloroform and the use of the various "spreaders," were: powdered pumice 99.8 per cent, filter paper 99.0–100.1 per cent, and filter-cel 100.1–100.8 per cent. Powdered pumice retains a comparatively large volume of chloroform and therefore a large volume of the solvent is necessary for extraction and washing; filter paper tends to form large masses, which may in some cases cause troublesome and incomplete extraction; and filter-cel was found to contain some chloroform-soluble material, which may account for the high results obtained. Filter-cel purified by removing the very small proportion of chloroform-soluble material is preferred next to finely powdered calcium carbonate, which is the best "spreader" and accordingly is specified in the proposed method. A recovery of 99.8 per cent is typical. In each case sufficient of the "spreader" was used with the emulsion sample to give practically a dry mixture in preparation for extraction of the oil with chloroform.

ABSORPTION OF OXYGEN BY COD LIVER OIL

It was shown experimentally that when 2.0000 grams of cod liver oil is dissolved in 100 cc. of chloroform and the chloroform is evaporated on a steam bath with the aid of a current of air, no increase in weight of the cod liver oil occurs. Since the proposed method directs weighing of the oil in a tared beaker, the conditions of drying that would cause a negligible or no increase in the weight of the oil were determined. The results of the various experiments are given in Table 1.

TABLE 1.—*Increase in weight of cod liver oil*

Conditions and period of heating

COD LIVER OIL*	NO HEATING, (ROOM TEMP.)	ELECTRIC OVEN		STEAM BATH, WITH CURRENT OF AIR	GAIN IN WEIGHT
		80° C.	100° C.		
grams	*days*	*hours*	*minutes*	*minutes*	*per cent*
1.2870	—	—	—	20	0.0
	—	—	—	50	0.07
	—	—	—	95	0.40
1.8715	—	—	20	—	0.0
	—	—	55	—	0.06
	—	—	95	—	0.08
2.0000	—	2	—	—	0.05
	—	48	—	—	3.5†
1.8050	3	—	—	—	0.8
	5	—	—	—	3.2†

* In an open 100 cc. beaker.
† Oil dark yellow and viscous.

It will be seen that the drying periods of 10 minutes on the steam bath with a current of air followed by 5 minutes at 100° C., as specified in the proposed method, is well within the safe limits to avoid an increase in weight or absorption of oxygen.

Besides influencing the weight of the oil, it is well known that exposure of fatty oils to atmospheric oxygen changes the analytical constants—the iodine value is decreased, the index of refraction increased, and the acidity may also increase. This is of extreme importance in case the analytical constants of the extracted oil are to be determined for the purpose of detecting inferior quality or adulteration of the cod liver oil used in preparing the emulsion.

PREPARATION OF THE EMULSION OF COD LIVER OIL

A sample of the emulsion in an open beaker loses weight upon standing at room temperature. This has a direct bearing on the cod liver oil content of an emulsion, because during its preparation the evaporation of some of the water may be sufficient to cause the finished emulsion to have a higher oil content than would be expected from the weights of the ingredients used. After various trials, the best procedure for preparing a small sample of a U.S.P. emulsion (about 4 grams), was found to be the English method, in which the acacia is dissolved in the water, the cod liver oil is added in small portions and thoroughly mixed to a thick, homogeneous emulsion, and the sirup is added and likewise mixed. In order to be able to determine definitely whether a loss in weight occurs due to the evaporation of some of the water during the preparation of an emulsion, the sample was prepared in a tared 100 cc. beaker. A tared stirring rod was used, the ingredients were weighed on a quantitative balance, a weighing buret was used for the oil, and the amounts of the ingredients were: acacia 0.5000 gram, water 1.5280 gram, cod liver oil 2.3915 grams, and sirup 0.5210 gram (alcohol and methyl salicylate were not used). The loss in weight during the preparation of the emulsion, or the difference between the sum of the weights of the ingredients and the weight of the finished emulsion, was found to be 6.7 per cent (this loss would vary in different lots). Doubtless a loss would also occur during the preparation of a large batch of emulsion. Such loss can not be readily and accurately determined, consequently the oil content of a large batch of an emulsion can not be accurately known from the weights of the ingredients.

Obviously, for the purpose of devising a method, the oil content of the emulsion should be accurately known; therefore, the entire amount of a small batch of emulsion (about 4 grams, prepared as given above) was used for each determination made by the collaborators and associate referee. The exact weight of the cod liver oil in each sample was known, and the oil was weighed by means of a weighing buret. In this experimental work, the exact weight of the oil alone is significant. Only the

approximate weight of each of the other ingredients was known and the total weight of the sample of emulsion was not taken into consideration.

Table 2 gives the results of collaborative study of the proposed method. The Associate Referee prepared each sample, and the entire sample was used for each determination. The collaborative samples, in form of an emulsion, contained besides the quantity of cod liver oil given, varying quantities of acacia, water, and sirup.

TABLE 2.—*Results of collaborative study of the proposed method*

COLLABORATOR	COD LIVER OIL		
	PRESENT*	FOUND	RECOVERY
	grams	*grams*	*per cent*
E. H. Berry	2.0165	2.0070	99.5
	2.0154	2.0047	99.5
	2.0530	2.0436	99.5
R. Jinkins	2.0720	2.0691	99.9
	2.0257	2.0177	99.6
H. O. Moraw	2.1697	2.1729	100.1
	2.0110	2.0146	100.2
R. S. Stanley	2.0250	2.0289	100.2
	2.0140	2.0124	99.9

* The U.S.P. cod liver oil was weighed by means of a weighing buret and incorporated in an emulsion.

EMULSION CONTAINING GLYCEROL AND ALCOHOL

Some emulsions on the market that contain cod liver oil also contain glycerol and alcohol. Experimental results obtained by the Associate Referee for carefully prepared samples of such an emulsion show that the proposed method will give good results. The four varied from 100.0 to 100.4 per cent recovery of the oil incorporated, about one-half as much glycerol as cod liver oil was used, and the alcohol content was about 7 per cent.

The method was published in *This Journal*, **22**, 96 (1939).

It is recommended[1] that the proposed method be adopted as a tentative method, and that the subject, in regard to the emulsion of cod liver oil, be closed.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, **22**, 57, 96 (1939).

REPORT ON OINTMENT OF MERCURIC NITRATE (CITRINE OINTMENT)

By H. O. MORAW (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

At the 1937 meeting the Associate Referee reported results of his investigation of possible methods and collaborative results on a proposed method. The method was based on separation of the mercury from the ointment base by 1+1 nitric acid digestion followed by acid permanganate digestion to insure that the mercury is in the bivalent state, eliminate oxides of nitrogen, and reduce organic matter; and titration of the mercury with standard thiocyanate. The results of the majority of the collaborators were within 3 per cent or less of the theoretical. The Committee recommended further study to improve the accuracy of the method.

Accordingly, the Associate Referee investigated the following points during 1938, and resubmitted the method to collaborators with a known sample. There were minor changes in the directions, but the principles of the method were the same as in 1937.

(1) Precautions necessary to insure the maintenance of an excess of permanganate during the acid permanganate digestion.

(2) Possible losses of mercury by volatilization during the digestions.

(3) Whether boiling during the 1+1 HNO_3 digestion is required or heating on steam bath is sufficient.

(4) Necessity of providing additional HNO_3 for the permanganate digestion.

The results of these investigations are shown in Table 1.

PURITY OF THE MERCURY

Two solutions designated A and B were prepared; redistilled mercury dissolved in nitric acid was used. Solution A consisted of 14.1050 grams of the mercury dissolved in 30 cc. of nitric acid and diluted to 1 liter. This was used chiefly for determining the purity of the mercury and checking on possible losses by volatilization. An average of four determinations showed the mercury to be 99.87 per cent pure. Solution B consisted of 31.1996 grams of mercury weighed in a tared glass-stoppered flask to which was added 44.57 grams of nitric acid (proportion required by the N.F. formula for preparing the mercuric nitrate used in making citrine ointment). After the reaction was completed in the open flask, it was stoppered and allowed to cool. It was found to weigh 70.9131 grams, which corresponded to 44.0 per cent crude mercury content. The mercury found in this solution by assay was 43.84–43.94 per cent, or an average of 43.88 per cent. Two uses were made of this solution, namely, mixing accurately weighed portions with appropriate quantities of nitrated lard to prepare assayable amounts of citrine ointment for use in checking the dependability of the proposed method, and for preparing the collaborative samples.

TABLE 1.—*Investigational results on known citrine ointment*

VARIATIONS FROM METHOD	NITRATED LARD*	MERCURIC NITRATE* SOLN. B	HG PRESENT	HG FOUND	RECOVERY	COMMENT
	grams	*grams*	*grams*	*grams*	*per cent*	
1. As proposed in 1937	5.5	0.9758	0.4282	0.4278	99.9	Satisfactory
2. As proposed in 1937 except no more HNO_3 added for $KMnO_4$ digestion		0.9758	0.4282	0.4195	98.0	Solution was the remaining half from first digestion in Determination 1
3. As proposed in 1937 except no more HNO_3 added for $KMnO_4$ digestion	5	0.7900	0.3467	0.3427	98.9	Half of HNO_3 digestion used
4. As proposed in 1937		0.7900	0.3467	0.3465	99.9	Remaining half of HNO_3 digestion from Determination 3
5. As proposed in 1937	3	0.7826	0.3435	0.3423	99.7	Satisfactory
6. 1st or HNO_3 digestion on steam bath	4	0.8431	0.3700	0.3554	96.1	Low result apparently due to failure to boil
7. 1+1 HNO_3 digestion in 200 cc. Erlenmeyer, gentle boiling	5	0.9194	0.4034	0.4016	99.5	Satisfactory
8. As proposed for 1937 HNO_3 digestion in Kjeldahl	4.2	0.7220	0.3168	0.3143	99.2	Satisfactory
9. 1+1 HNO_3 digestion in 200 cc. Erlenmeyer	4.0	0.9149	0.3934	0.3932	100.0	Satisfactory
10. 1+1 HNO_3 digestion in 200 cc. Erlenmeyer	4.0	1.5882	0.6829	0.6813	99.8	Satisfactory
11. 1+1 HNO_3 digestion in 200 cc. Erlenmeyer. 15 cc. H_2SO_4 added to half of soln and digested to fuming	5.0	0.9194	0.4034	0.3921	97.1	This experiment was on half of the 1+1 HNO_3 digestion from Determination 7.

* Same as required for the N.F product; they were mixed in the digestion flask by gentle warming and then used for assays.

DISCUSSION OF ABOVE EXPERIMENTAL RESULTS

Heating the 1+1 nitric acid digestion on the steam bath apparently does not effect complete extraction of the mercury. Gentle boiling seems to be necessary. The additional nitric acid specified in the method for the potassium permanganate digestion is necessary to effect complete recovery. The use of the Kjeldahl flask for the 1+1 nitric acid digestion does not seem to be necessary, since the recoveries were equally as good with the 200 cc. Erlenmeyers. From the recoveries obtained it may be assumed that there is no detectable loss by volatilization during either of the digestions as directed in this method.

TABLE 2.—*Volatility of mercury from $Hg(NO_3)_2$ solution*

(No organic matter present)

DET. NO.	SAMPLE AND TREATMENT	HG PRESENT	HG FOUND	RECOVERY
		grams	*grams*	*per cent*
1	25 cc. Soln A in 500 cc. Kjeldahl +40 cc. 1+1 HNO_3, digested 2 hrs. Final volume, 40 cc.	0.3521	0.3517	99.9
2	25 cc. Soln A in 500 cc. Kjeldahl +40 cc. 1+1 HNO_3, digested 2 hrs. Final volume, 40 cc.	0.3521	0.3502	99.5
3	25 cc. Soln A in 250 cc. lipped Erlenmeyer+40 cc. 1+1 HNO_3 digested 1 hr. Final volume 40 cc.	0.3521	0.3522	100.0
4	25 cc. Soln A in 250 cc. lipped Erlenmeyer+40 cc. 1+1 HNO_3 digested 1½ hrs. Final volume 15 cc.	0.3521	0.3522	100.0
5	25 cc. Soln A+40 cc. 1+1 HNO_3 +15 cc. H_2SO_4 digested to fuming	0.3521	0.3543	100.7*

* End point not normal.

Experiment 11 (Table 1) shows one of the conditions (not applicable in this method) under which there is a slight loss of mercury by volatilization. In this experiment half of the nitric acid digestion from Experiment 7 was digested to fuming after sulfuric acid had been added, and the recovery was only 97.1 per cent compared with 99.5 per cent in Experiment 7, which was the other half of the solution treated according to the method. This table presents the results of 7 determinations averaging 99.7 per cent recovery after the samples of authentic citrine ointment had been digested about 1½ hours with 1+1 nitric acid and filtered, and the filtrate digested 30–45 minutes with HNO_3-H_2SO_4-$KMnO_4$. The recovery was about the same whether the first digestion was conducted in 500

cc. Kjeldahl or 200 cc. Erlenmeyer flasks with short-stemmed funnels in the necks. In all cases the second digestions were made in open 500 cc. Erlenmeyer flasks.

Solutions of mercuric nitrate in nitric acid and in nitric-sulfuric acids were digested (in the absence of organic matter) in 500 cc. Kjeldahl and 200 cc. Erlenmeyer flasks from volumes starting with 65 cc. of 1+2 nitric acid to final volumes of 40 cc. and 15 cc. Complete recovery of the mercury was obtained as shown in Table 2. Experiment 6 in this table shows the result of digesting to fuming after adding sulfuric acid. There was no organic matter present in this case, and although not conclusive the result, when considered with Experiment 11 in Table 1, suggests that the loss in the latter case was connected with the organic matter present.

TABLE 3.—*Collaborative results*

COLLABORATOR	OMITTING TEST FOR COMPLETE EXTRACTION OF HG FROM FAT		ADDING TEST FOR COMPLETE EXTRACTION OF HG FROM FAT	
	HG FOUND	RECOVERY ON 7.28% PRESENT	HG FOUND	RECOVERY ON 7.28% PRESENT
	per cent	*per cent*	*per cent*	*per cent*
E. H. Grant, U. S. Food & Drug Adm., Boston			7.39	101.5
			7.34	100.8
C. B. Stone, U. S. Food & Drug Adm., Cincinnati	7.33	100.7	7.30	100.3
	7.33	100.7	7.30	100.3
Rupert Hyatt, U. S. Food & Drug Adm., Cincinnati	7.44	102.3	7.38	101.4
	7.42	101.9	7.39	101.5
K. L. Milstead, U. S. Food & Drug Adm., St. Louis	7.37	101.2	7.29	100.1
	7.35	101.0	7.31	100.4
Wm. F. Reindollar, Maryland Bur. Chemistry, Baltimore	7.20	98.9	7.14	98.1
	7.03*	96.6*	6.88*	94.5*
	7.26	99.7	7.24	99.5
E. H. Berry, U. S. Food & Drug Adm., Chicago	7.22	99.2	7.22	99.2
	7.26	99.7	7.22	99.2
H. O. Moraw, U. S. Food & Drug Adm., Chicago	7.29	100.1	7.26	99.7
	7.30	100.3	7.26	99.7
L. E. Warren, U. S. Food & Drug Adm., Washington	7.39	101.5	7.38	101.4
	7.40	101.6	7.39	101.5
			7.43	102.0
			7.42	101.9
			7.39	101.5
Average of all results	7.31	100.4	7.30	100.2
* Average omitting lowest.	7.32	100.6	7.32	100.5

The method submitted to collaborators was published in *This Journal*, 22, 96 (1939).

COMMENTS BY COLLABORATORS

E. H. Grant.—(Submitted too late for publication 1937): The directions should call for preparing the 3% peroxide from the 30% article, The acetanilid present in the commercial product may form a yellow color, which interferes with the end point.

Rupert Hyatt.—The test for complete extraction was practically negligible, and does not appear to be necessary. Shorter heating periods, i.e., boiling 5 minutes with the 1+1 HNO_3 and 10 or 15 minutes for the $KMnO_4$ digestion should suffice. Stone and I mixed our samples before beginning the analysis. Since we obtained checks on ourselves, the difference is apparently due to sample or personal equations.

K. L. Milstead.—By trial I found that the test for complete extraction required about the same volume of 0.1 *N* NH_4CNS whether diluted to 200 cc. and only 100 cc. used or whether the entire test is carried through the $KMnO_4$ digestion, i.e., about 1 minim or 0.05 cc. at most. A blank on the reagents required only a fraction of a drop. Even when varying amounts of sample were used, the test for complete extraction required the same volume of thiocyanate. I believe the test for complete extraction is unnecessary and the additional titration is not due to Hg.

E. H. Berry.—On testing for complete extraction 0.1 cc. was required on one determination and none on the other.

Wm. F. Reindollar.—Although the amount of permanganate consumed seems inordinately large, the method presents no difficulties when followed closely.

SUMMARY

Results are submitted showing an average recovery of 99.7 per cent of the mercury in seven experiments by applying the proposed method to authentic individual assayable quantities of citrine ointment, each prepared separately from accurately weighed amounts of mercuric nitrate of known mercury content and mixed with nitrated lard; each of these was prepared as required by the N.F. for the official citrine ointment. Attention is directed to the fact that in Experiments 1 and 2, 3 and 4, 7 and 11, one-half of the nitric acid extract was used for completing the determination as directed by the method and the other half used for a modified method. In this way the effect of the variation could be attributed to no other cause than the variation itself.

These results should remove doubt concerning the supposed pitfalls logically applicable to mercury digestions, such as incomplete extraction of the mercury from the ointment base, loss of mercury by volatilization, and titration of all the mercury.

The finished weight of the batch of ointment prepared for collaborative work was 280.41 grams and it contained 20.411 grams of mercury or 7.28 per cent. The majority of the collaborative results did not vary more than ±0.1 per cent from this amount, and the average of 14 results, 7.31 per cent, is in good agreement. Whether the variations indicate non-uniformity of the collaborative sample or reflect the personal factor can not be decided. If it is conceded that analytical accuracy may not be

attained in preparing large amounts of a product such as the 280 grams for collaborative study and that the true mercury content was not 7.28 per cent, it nevertheless appears that the uniformity of the collaborative results is reasonably good and that they indicate approximately the correct amount.

Two of the collaborators, Milstead and Hyatt, do not believe the test for complete extraction is necessary and Milstead points out that the same volume of 0.1*N* ammonium sulfocyanate is required for the test irrespective of size of sample, and about the same for a blank. It was the belief of the Associate Referee based on 1937 tests that this test was unnecessary. However, since the personal equation may enter into application of the method and it is the analyst's responsibility to insure complete recovery, this test was incorporated in the 1938 directions. Further reflection on this point in connection with comments by the collaborators indicates that the titration from the test should not be added to the main titration unless it is greater than a blank titration.

It is recommended[1] that the method be adopted as a tentative method with a view to making it official and that the subject be closed.

No report on rhubarb and rhaponticum was given by the associate referee.

No report on theophylline sodium salicylate was given by the associate referee.

REPORT ON SULFANILAMIDE

By EDWARD M. HOSHALL (U. S. Food and Drug Administration, Baltimore, Md.), *Associate Referee*

The widespread use of sulfanilamide (para-aminobenzenesulfonamide) in the treatment of various bacterial infections in humans and the dangers associated with its indiscriminate use make it desirable to have an accurate method for its determination in tablets and to a lesser extent in the other forms in which it may be dispensed. At the present time practically all the product is available only in tablet form, either with suitable tablet excipients or with equal quantities of sodium bicarbonate.

Methods for the analysis of sulfanilamide may be based on any one of the following general types:

1. Determination of carbon, hydrogen, and nitrogen by combustion analysis.
2. Determination of nitrogen by the Kjedahl method.[2]
3. Determination of sulfur by Messenger's[3] or other methods.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, **22**, 58, 96 (1939).
[2] *Methods of Analysis, A.O.A.C.*, 1935, 23.
[3] *Quart. J. Pharm. Pharmacol.*, **9**, 560 (1936).

4. Determination of the nuclear-attached amino group by diazotization and observing the end point with starch-iodide paper,[1] or diazotization and subsequent coupling with organic reagents to form azo dyes, which are then determined colorimetrically.[*,2,3]
5. Determination by mercuration.[4]
6. Determination by bromination of the nucleus.[5,6]
7. Determination of the sulfonamido group.[5]

PART I

EXPERIMENTAL

Pure sulfanilamide, M.P. 166.0° C. was obtained. It was twice recrystallized from boiling alcohol and dried under vacumn and finally over sulfuric acid. The melting point remained constant. It was then examined for contaminants by the tests proposed by the A. M. A. Chemical Laboratory[1] and found to conform to their standards of identity and purity in all respects. Nitrogen determined by the A.O.A.C. Kjeldahl method was equivalent to a purity of 99.8 and 99.9 per cent of sulfanilamide. This material was used as a standard in all the experimental work and in the preparation of the collaborative samples.

The first three general methods were adjudged to be lacking in specificity and accordingly no work was carried out on these methods.

Diazotization with 0.1 *N* sodium nitrite in the presence of acid and determination of the end point with starch-iodide test paper by the recommended A. M. A. Laboratory Method[1] was investigated. The results obtained are shown in Table 1.

TABLE 1.—*Results by A.M.A. laboratory method*

TAKEN	FOUND	RECOVERY
gram	*gram*	*per cent*
0.0700	0.0703	100.43
0.0700	0.0701	100.14
0.2000	0.1985	99.25
0.2000	0.1992	99.60
0.5000	0.4955	99.10
0.5000	0.4962	99.24
0.7000	0.6930	99.00
0.7000	0.6922	98.89

The method is simple and accurate, and the results are in satisfactory agreement. The objections are that the method is not specific, the sodium nitrite solution must be very frequently standardized, the reaction is slow towards the end, and experience is necessary to properly interpret the end point.

[1] *J. Am. Med. Assoc.*, 109, 359 (1937).
[*] *Quart. J. Pharm. Pharmacol.* 9, 560 (1936).
[2] *J. Biol. Chem.*, 122, 263 (1937).
[3] *Lancet*, 232, 194 (1937).
[4] *Ibid.*, 195.
[5] *Z. Anal. Chem.*, 108, 396 (1937).
[6] Minutes of the Combined Contact Committee Meeting of March 28–29 1938. Item No. 12.

Diazotization and coupling with dimethyl-α-naphthylamine in acid solution to produce a purple-red dye and measurement of the intensity of the color by means of comparison with a standard, as in the excellent micro method of E. K. Marshall, Jr.,[2] was used quite extensively in this laboratory for the determination of the drug in a liquid preparation, and satisfactory results were obtained (Table 2). A similar method* except that α-naphthylamine was used as the coupling agent was found to be less satisfactory. The use of β-naphthol in alkaline solution as the coupling agent according to the method of Fuller[3] was tried, and generally unsatisfactory results were obtained. The colorimetric methods are essentially micro methods, and for that purpose are excellent, especially the Marshall method. As general routine methods they are impracticable in that special organic reagents and apparatus to protect the reagents from the air and light are required, a blank determination on a "known" must be run concurrently with the sample, and finally a precision colorimeter is necessary. The overall accuracy of the Marshall method appears to be ±2 per cent.

TABLE 2.—*Results by Marshall method*

TAKEN (BEFORE DILUTION)	COLORIMETER READINGS	FOUND (CALCULATED)	RECOVERY
gram	*gram*	*per cent*	*per cent*
0.5	10.1	0.505	101
0.5	10.2	0.510	102
1.0	9.9	0.99	99
1.0	9.8	0.98	98

Mercuration with mercuric nitrate in slightly acid solution[4] was investigated. The resultant complex insoluble precipitate was dried and weighed, and although it was found possible to control the composition of the precipitate by following an exact procedure, it was necessary to assign an empirical factor to convert the weight of the precipitate to sulfanilamide. If necessary, the sulfanilamide can be recovered from the complex by precipitating the mercury with hydrogen sulfide, filtering off the mercuric sulfide, and evaporating the filtrate at about 70° C. with the aid of a fan. The residue, impure sulfanilamide, is then recrystallized from hot alcohol. Recoveries are not quantitative.

The direct bromination of the nucleus by a method similar to that used for the determination of acetanilid in *Methods of Analysis, A.O.A.C.*, 1935, appeared theoretically practical for sulfanilamide. Investigation, however, disclosed that low results, Table 3, were obtained, the formation of the dibromo derivative proceeding quite slowly towards the end.

Attention was next directed to an indirect bromination, namely that of adding an excess of a 0.1 *N* potassium bromide-bromate solution, acidifying, allowing to stand, then adding potassium iodide, and finally titrating the liberated iodine with 0.1 *N* sodium thiosulfate, using starch

* *Quart. J. Pharm. Pharmacol.* 9, 560 (1936).

as indicator. Two papers proposing this method have since appeared in the literature.[5,6] Preliminary work on this method consisted of ascertaining the effect of the several factors that might influence the accuracy of the method. These factors and the results obtained when they were varied are shown in Table 4. Unless otherwise stated the sample consisted in all cases of 0.15 gram of recrystallized sulfanilamide.

TABLE 3.—*Results by A.O.A.C. acetanilid method*

TAKEN	FOUND	RECOVERY
gram	*gram*	*per cent*
0.2000	0.1948	97.4
0.2000	0.1930	96.5
0.5000	0.4885	97.7
0.5000	0.4877	97.5

TABLE 4.—*Factors influencing indirect bromination method*

EFFECT OF VARYING THE EXCESS OF BROMINE PRESENT

0.1 *N* BROMINE PRESENT	0.1 *N* BROMINE REQUIRED	EXCESS BROMINE PRESENT	SULFANILAMIDE
cc.	*cc.*	*per cent*	*per cent*
34.85 (theory)	34.54	1	99.13
38.86	34.96	11.2	100.34
48.58	35.02	28.7	100.51
58.29	35.06	66.3	100.62
97.15	35.54	173	102.00

EFFECT OF TIME OF BROMINATION

50.00 cc. 0.1 *N* bromine added

TIME OF STANDING	SULFANILAMIDE
	per cent
About ½ min.	100.39
5 min.	100.51
15 min.	100.47
45 min.	100.51
12 hours	104.58

EFFECT OF VOLUME OF LIQUID DURING BROMINATION

LIQUID PRESENT		TOTAL VOLUME	SULFANILAMIDE
cc.		*cc.*	*per cent*
10 cc. HCl+10	0.5 *N* Br	20	100.82
+50	0.1 *N* Br	60	100.93
25 cc. H_2O+10	HCl		
+50	0.1 *N* Br	85	101.20
65 cc. H_2O+10	HCl		
+50	0.1 *N* Br	125	102.40

EFFECT OF LIGHT ON BROMINATION

CONDITION DURING BROMINATION—	SULFANILAMIDE FOUND	SULFANILAMIDE RECOVERY
	gram	*per cent*
In dark	0.1507	100.48
Diffused daylight	0.1508	100.56
Bright sunlight	0.1513	100.85

EFFECT OF TEMPERATURE ON BROMINATION

TEMPERATURE DURING BROMINATION	SULFANILAMIDE FOUND	SULFANILAMIDE RECOVERY
	gram	*per cent*
Room temp. (24° C.)	0.1507	100.47
Room temp. (24° C.)	0.1508	100.53
Refrigerator (8° C.)	0.1504	100.27
Refrigerator (8° C.)	0.1503	100.20
Ice-water (1.5° C.)	0.1503	100.20
Ice-water (1.5° C.)	0.1502	100.13

A comparison of the preliminary results obtained shows that under similar conditions check results were obtained and were reproducible. Within normal working limits none of the factors investigated appreciably affected the results obtained. In general the results are above the theoretical, and this was first attributed to the almost inevitable loss of small amounts of bromine when the potassium iodide solution was introduced. Later work indicated that the excess found was probably due to a retention of bromine by the formation of a sulfondibromamide with the sulfanilamide. It may also be noted that a difference of 0.1 cc. of 0.1 *N* thiosulfate in the titration made a difference of ± 0.3 per cent in the per cent recovery figure.

Despite the fact that the indirect bromination method yielded somewhat high results, it is believed that it should be collaboratively studied, especially as it was more accurate than the colorimetric methods, which were the only other methods available when this work was begun. The following collaborative samples were prepared:

Sample A.—Consisted of 7.3 grain tablets of sulfanilamide prepared commercially under the supervision of the Associate Referee. The tablet mixture contained 69.5% and the dried granulations contained 68.4% of sulfanilamide, the balance being composed of starch and talc. The actual sulfanilamide content of the tablets as determined from the Kjeldahl nitrogen content was 68.6 per cent. 5000 of the tablets were aged and dusted, and portions were placed in clean containers for the collaborators.

Sample B.—Purified sulfanilamide (see first page this report).

Sample C.—Consisted of a powder composed of the ingredients listed on p. 753.

Nitrogen was determined by the Kjeldahl method on the mixture and also on the gelatin component. After due correction for gelatin nitrogen, the nitrogen was equivalent to 47.48 per cent sulfanilamide.

	per cent
Sulfanilamide	47.37
Talc	11.70
Gelatin	2.92
Lactose	5.85
Chalk	5.85
Gum acacia	2.92
Calcium phosphate	5.85
Potato starch	11.70
Kaolin	5.85
Total	100.01

Each component was ground to pass a 60-mesh sieve, then weighed out and mixed. The mixture was psssed through a 60-mesh sieve three times, subdivided by quartering and requartering, then placed in clean containers and sealed.

Collaborators were supplied with portions of each of the above samples and were requested to make duplicate determinations by Method 1 as follows:

METHOD I

Place a portion of the sample containing 0.1–0.3 gram of sulfanilamide, in a 500 cc. glass-stoppered iodine absorption flask,* add about 25 cc. of water and sufficient standard bromide-bromate solution (0.1 *N* or 0.5 *N*) (0.5 *N* bromide-bromate solution, *Methods of Analysis, A.O.A.C.*, 1935, 543. 0.1 *N* bromide-bromate solution, *Ibid.*, 551) to ensure a 10 to 50 per cent excess of Br. Add rapidly 10 cc. of HCl and immediately insert the stopper. Swirl the flask, and place in the dark about 5 minutes. Remove the stopper just sufficiently to introduce quickly 10 cc. of 10 per cent KI solution, taking care that no Br vapors escape, and immediately stopper the flask. Shake thoroughly, remove the stopper, and rinse it and the neck of the flask, receiving the washings in the flask. Add about 150 cc. water, and then titrate the liberated I with 0.1 *N* $Na_2S_2O_3$ solution, using starch indicator.

1 cc. of 0.1 *N* bromide-bromate solution = 0.004305 gram of sulfanilamide.

NOTES

(1) If carbonates are present, make the sample slightly acid and allow the CO_2 to pass off; then make slightly alkaline with NaOH (1 + 10), and proceed as directed previously.

(2) If difficulty is experienced by loss of Br vapors, cool the stoppered flask before the addition of the KI solution.

The collaborators' results are reported in Table 6.

COLLABORATORS

Charles F. Bruening, U. S. Food and Drug Adm., Baltimore.
Donald C. Grove, U. S. Food and Drug Adm., Washington.
Maurice Harris, U. S. Food and Drug Adm., Houston.
The Associate Referee.
William F. Reindollar, State Department of Health, Baltimore.
S. Reznek, U. S. Food and Drug Adm., New York City.
Phileas A. Racicot, Dept. of Public Health, Food and Drug Div., Boston.

* The use of an iodine absorption flask was not specified in the method sent to the collaborators.

TABLE 6.—*Collaborative results*

COLLABORATOR	SULFANILAMIDE		
	SAMPLE A	SAMPLE B	SAMPLE C
	per cent	*per cent*	*per cent*
1	70.71	101.9	49.91
	70.77	101.9	49.84
2	69.72	100.24	48.96
	69.83	100.39	48.96
3	70.68	100.86	49.36
	70.44	100.90	49.06
4	69.88	100.65	48.52
	69.90	100.51	48.86
5	68.42	99.26	48.29
	69.02	99.55	47.70
6	70.6	100.0	48.6
	70.9	100.3	48.6
7	73.0	101.4	55.9
	73.4	100.6	55.1

COMMENTS OF COLLABORATORS (ABSTRACTED)

Bruening.—Special iodometric flasks were used, so designed that the 10 cc. of KI solution can be placed in the reservoir above the stopper and as the stopper is lifted the KI solution is drawn into the flask and no bromine vapor is lost. (Also commented on high results.)

Grove.—Suggested iodine flasks and commented on high results obtained.

Harris.—It is noted that the brominated sulfanilamide absorbed iodine during the back titration with thiosulfate, as evident by the absence of a pure white precipitate. If a small amount of alcohol is added near the end point, it will displace the iodine from the surface. The high results indicated for Sample B may be due to undesired substitution and can be avoided by brominating at low range temperatures as 0°–5° C. The selected temperature should also insure quantitative substitution. (An iodine absorption flask was also suggested.)

Reindollar.—The blue color tends to return after titration flasks have stood awhile. This does not interfere. During the 5 minute reaction period the flasks were placed in ice water . . . ."

Reznek.—Noted slightly high results.

Racicot.—Suggested that a cork-stoppered Erlenmeyer flask provided with dropping funnel with cock be used to prevent loss of bromine vapor.

DISCUSSION

It is evident from the results of the collaborators, Table 6, that the method yields somewhat high results in the case of all three samples. Investigation of several factors that might be conducive to the production of such excesses has disclosed no points whereby the method can be modified so that quantitative yields might be obtained. As previously stated the excess is apparently due to a reaction of the bromine with the sulfonamido group and the formation of a sulfondibromamide, the bromine of which is not wholly liberated under the conditions of the test.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method described in this report be adopted as an alternative tentative method (see Part II).

(2) That due notice be made of the tendency of the method to yield slightly high results.

PART II

EXPERIMENTAL

It is generally agreed that the sulfonamido group ($-SO_2NH_2$) is the therapeutically important group in sulfanilamide and its many derivatives, which are being made commercially available. It would appear that a method based on the determination of this group would be more desirable and certainly more specific than any of the methods discussed in Part I.

This group is, in general, resistant to reduction and to hydrolysis, and due to its stability does not enter into many reactions with ordinary reagents. In this connection the work of Chattaway[2] was reviewed. This worker found that some sulfonamides react quantitatively with hypobromous acid and form relatively stable sulfondibromamides. These compounds are soluble in organic reagents and on reacting with strong acids liberate the bromine, which then can be determined iodometrically. The fact that this method has failed to yield quantitative results to date when applied to sulfanilamide may be explained by the fact that Chattaway used benzenesulfonamide and toluenesulfonamide instead of the amino derivative. Sulfanilamide reacts with the hypobromous acid in a vigorous manner, and unless cooled, oxidation and even carbonization may occur. With suitable precautions it was found possible to prepare the sulfondibromamide, extract, and determine the amount present by iodometric titration. Poor yields were obtained. In an effort to increase the yield the sulfanilamide was acetylated and the acetyl derivative was then treated by the following outlined method:

A 0.25 gram sample of sulfanilamide was acetylated in a separator by adding an excess of acetic anhydride, shaking vigorously, and allowing to stand until clumps of needle-like crystals separated. The separator and its contents were cooled to about 2° C., and 25 cc. of a solution of hypobromous acid* cooled to 2° C. was added. The separator was shaken for 5 minutes and frequently immersed in ice water. The sulfondibromamide was then extracted with several portions of chloroform, each portion being washed with 5 cc. of the hypobromous acid solution. The combined chloroform extracts were filtered through cotton and evaporated at room temperature with the aid of a fan. The residue was dried over sulfuric acid and weighed as the dibrom derivative. It may be taken up in dilute alkali, transferred

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 58 (1939).
[2] *J. Chem. Soc.*, 87, 145 (1905).
* Prepared by shaking Br with precipitated HgO suspended in water.

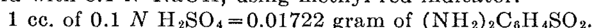
to an iodine absorption flask, the bromine determined iodometrically by the addition of potassium iodide, acid, and water, and the liberated iodine titrated with thiosulfate, starch being used as indicator.

By the use of this method it was possible to obtain yields up to 80 per cent of the sulfanilamide added. Due to the numerous steps in this method, the care that must be exercised in handling the sulfondibromamide, and the poor yields obtained, no further work was done on this method. However it is contemplated that further work will be done along these lines, since in addition to being useful to separate the sulfanilamide from other drugs the method is quite specific for sulfanilamide and many of its derivatives.

A somewhat simpler method, which could be applied to the analysis of the sulfonamido group, is that of hydrolysis, whereby the ammonium sulfate formed when sulfuric acid is used as the hydrolyzing agent is determined by making alkaline, distilling, and determining the ammonia in the distillate. Preliminary work on this method was confined to determination of optimum conditions for hydrolysis of the sulfanilamide, and the results are embodied in Method II.

METHOD II

Place on a 9 cm. folded filter paper in a funnel a portion of the sample containing about 0.5 gram of sulfanilamide. Wash the soluble portion with a fine stream of acetone into a 250 cc. flask, using a total of about 25 cc. of acetone, Test for complete extraction by evaporating a small portion of the washings. Immerse the flask in a water bath at about 70° C. until the acetone has been evaporated and its odor is no longer perceptible. Remove from bath and add 10–12 cc. of 75% (by volume) H_2SO_4. Connect the flask to a reflux condenser with water jacket, add a few glass beads, and boil slowly for 30 minutes. Wash down the condenser with water, make the liquid in the flask to about 100 cc. with water, add an excess of 50% alkali, distil, and collect the ammonia in the distillate in an excess of 0.1 *N* H_2SO_4. Titrate the excess acid with 0.1 *N* NaOH, using methyl red indicator.



1 cc. of 0.1 *N* $H_2SO_4 = 0.01722$ gram of $(NH_2)_2C_6H_4SO_2$.

After the preliminary work on this method had been carried out, and a short time before the 1938 A.O.A.C. Meeting, a few collaborators were requested to make duplicate determinations on Samples A, B, and C by Method II. In spite of the last-minute request four of the collaborators very kindly responded. Their collaborative results appear in Table 7.

The collaborators were Charles F. Bruening; Llwellyn H. Welsh, U. S. Food and Drug Adm. Baltimore; William F. Reindollar, and the Associate Referee.

COMMENTS OF COLLABORATORS (ABSTRACTED)

Bruening.—The end point was somewhat difficult to ascertain; the distillates were titrated until practically all the red color disappeared.

Welsh.—Do not believe necessary to keep temperature below 100° C. in removal of acetone.

Reindollar.—In spite of the approximate results obtained (due to interruptions

during analysis), I feel that with due attention and experience the method is capable of yielding better results, and is certainly more specific than the bromination method.

TABLE 7.—*Collaborative results by Method II*

COLLABORATOR	SULFANILAMIDE		
	SAMPLE A	SAMPLE B	SAMPLE C
	per cent	*per cent*	*per cent*
1	69.1	100.0	48.0
	69.5	100.2	47.9
2	69.11	99.88	47.87
	68.63	100.08	47.96
3	68.71	99.98	47.70
	68.86	100.01	47.53
4	67.91	99.35	48.05
5			
Averages:	68.83	99.93	47.86

DISCUSSION

The results obtained by Method II, Table 7, indicate a more satisfactory agreement with the known sulfanilamide content of the collaborative samples than do the results obtained by Method I, Table 6. In the second method the results are slightly high, although the average appears to be within the analytical error for a determination of this nature.

Method II is simple and short and requires reagents and apparatus found in all analytical laboratories. It has received no fundamental adverse criticism from collaborators.

Due to the late date at which the method was developed, it has been studied by only four collaborators. In spite of this fact the Associate Referee believes that no additional work is necessary, first because the method is direct, and secondly because it is believed that this drug will be included in the forthcoming U.S.P. XI Supplement No. 2, which will remove it from the scope of the A.O.A.C. unless no assay method is provided by the U.S.P. XI.

It is recommended,[1] therefore, that the method described in Part II of this report be adopted as a tentative method.

REPORT ON MANDELIC ACID

By H. G. UNDERWOOD (U. S. Food and Drug Administration Chicago, Ill.), *Associate Referee*

The study of mandelic acid was undertaken in accordance with the recommendation of Subcommittee B. It appeared to be desirable to investigate the properties of mandelic acid and to develop a method for

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 58 (1939).

its determination in typical market preparations. This product and its salts have been used recently for the treatment of infections of the urinary tract.

The results of collaborative study and recommendations are included in this report.

REVIEW OF LITERATURE

Mandelic acid, $C_6H_5CH(OH)COOH$, due to the presence of an asymmetric carbon atom in the molecule, exists in three definite forms. The synthetically prepared compound is the racemic or (dl) mandelic acid, and hence is optically inactive. Mandelic acid is a white, crystalline compound, which gives colorless and clear solutions in water, alcohol, and alkali. Its melting point has been reported by Claisen[1] to be 118° C. and by others to be 118–120° C.[2] The solubility is 15.95 grams at 20° C. in 100 cc. of water; 53.6 grams dissolve in 100 cc. ethyl alcohol at 16.5° C. It is also soluble in ether and other organic solvents. The pure acid is slowly decomposed by light, with the liberation of benzaldehyde, and should, therefore, be stored in the dark, or in suitable light-proof bottles. Mandelic acid reacts readily with basic substances to produce salts or "mandelates."

The sodium, ammonium, calcium, and magnesium salts as well as compounds of mandelic acid with ethanolamine, ethylene diamine, and hexamethylenetetramine have been reported of value in mandelic acid therapy. In general, the drug is encountered in tablet form and solutions, usually as a mandelate.

EXPERIMENTAL

Several qualitative tests for mandelic acid are reported in the literature. An aqueous solution of mandelic acid gives the test for an alpha-hydroxy acid with ferric chloride.[2] Mandelic acid when treated with sulfuric acid, under certain conditions of concentration, gives a purple coloration and the odor of benzaldehyde.[3] The Associate Referee requested the collaborators to report on these tests to determine whether they are suitable for the identification of mandelic acid. The collaborators were also requested to report on the melting point by the U.S.P. method. The mandelic acid submitted for the above tests titrated 99.9 per cent.

F. Reimers[4] reports that mandelic is quantitatively isolated from hydrochloric acid solution by 5 or 6 extractions with a double volume of ether. The ether is evaporated on a water bath and the residue titrated with 0.1 *N* sodium hydroxide, phenolphthalein being used as indicator. E. M. Hoshall[5] of the Baltimore Station, U. S. Food and Drug Administration, also reports practically quantitative recovery by ether extraction and titration.

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[1] *Ber.*, **10**, 847 (1877).
[2] *J. Am. Med. Assoc.*, **109**, 24, 1989 (1937).
[3] Mulliken, Vol. 1.
[4] *Dansk Tids Farm.*, **12**, 25–32 (1938).
[5] Private communication.

Collaborative results on mandelic acid

COLLABORATOR	SAMPLE I		ELIXIR				M. P. MANDELIC ACID U. S. P. METHOD
	TABLET METHOD		TITRATION WITH 0.5 N NAOH		DILUTION METHOD TITRATION WITH 0.1 N NAOH		
	G./100 CC.	% RECOVERY	G./100 CC.	% RECOVERY	G./100 CC.	% RECOVERY	
Theoretical	90.0		40.0		40.0		
I. S. Shupe Kansas City	90.0 89.9	100.0 99.9	39.8 39.6	99.5 99.0			118.5–119° C.[1]
J. Carol Cincinnati	89.6 89.6	99.6 99.6	39.9 40.0 39.7	99.8 100.0 99.3			116 –118° C.
R. Hyatt Cincinnati	89.85 89.85	99.8 99.8	39.9 40.0	99.8 100.0	40.0 40.0	100.0 100.0	118 –119° C.
C. B. Stone Cincinnati	89.6 89.6	99.6 99.6	40.05 40.05	100.1 100.1	40.0 40.1	100.0 100.3	
L. D. Seif* Cincinnati (N. C. Anewalt, Analyst)	87.43 88.19	97.1 98.0			39.4 39.0	98.5 97.5	118 –119° C.
G. M. Johnson Minneapolis	89.71 89.82 89.79 89.71	99.7 99.8 99.8 99.7	39.67 39.63 39.70 39.79	99.2 99.1 99.3 99.5			
H. G. Underwood Chicago	89.9 90.0 89.7	99.9 100.0 99.7	39.84 39.84	99.6 99.6	40.0 40.0	100.0 100.0	118 –119° C.
Average, all values	89.54	99.5	39.83	99.59	39.81	99.54	

* Wm. S. Merrell Co., all other collaborators U. S. Food & Drug Adm.
[1] On the same thermometer benzoic acid melted at 121.5–122° C.

The Associate Referee tried a mixed solvent of chloroform and ether (2+1) and found it satisfactory for the extraction of mandelic acid from solution. The chloroform-ether solvent, in contrast to ether, makes for more convenient manipulation, since it forms the lower layer in the separator. Since mandelic acid titrates readily, no attempts were made to determine it gravimetrically. Furthermore, mandelic acid sublimes slowly at 100° C., and any gravimetric method would necessitate drying at a lower temperature or in a desiccator.

Two samples were prepared and submitted to collaborators with the proposed methods. The first consisted of a mixture of mandelic acid (90 per cent) and starch. The second sample, an elixir, was prepared to represent a liquid preparation and was compounded as follows:

Mandelic acid	400 grams
NH_4OH solution to neutral.	
Tincture sweet orange peel	20 cc.
Sirup	100 cc.
Alcohol	200 cc.
Water to make 1000 cc.	

The methods were published in *This Journal*, 22, 98 (1939). The results are given in the table.

COMMENTS BY COLLABORATORS

I. S. Shupe.—Tests on both methods showed incomplete extraction with the seventh portion of solvent. The eighth and ninth extractions still showed traces of mandelic acid, but the titrations were insignificant. The qualitative tests responded as described in your method. The qualitative color test with sulfuric acid did not respond when too small an amount of mandelic acid was used.

G. M. Johnson.—(a) A bright yellow color was obtained. (b) A purple tint was obtained. Neither of the above seems to be a very conclusive test.

L. D. Seif.—The qualitative tests were satisfactory, although the colors produced were not very intense. The bright yellow color produced with $FeCl_3$ was slightly more intense than that produced by adding the same amount of $FeCl_3$ to distilled water. We have tried a qualitative test by melting mandelic acid and hydroquinone together and dissolving in 10 per cent NaOH. The color produced here was no more definite than the color produced in the other tests.

R. Hyatt.—The qualitative tests were observed to give the results indicated in the description. In (b) the development of the purple color was slow if the solutions were mixed. If the solutions were not mixed, the color development was quicker and just as characteristic. The tests appear to be satisfactory.

C. B. Stone.—The qualitative tests gave the results described in the methods. If too small a sample was used or if the solutions were rapidly mixed, the purple color did not form in the test with H_2SO_4. However, in both cases, the odor of benzaldehyde was noted.

DISCUSSION

The results obtained by the collaborators on the two samples are quite satisfactory. About seven extractions are necessary to remove the mandelic acid, this number being necessitated, no doubt, by the relatively high solubility of mandelic acid in water.

The average value for the melting point of mandelic acid as determined by the U.S.P. method is 118°–119° C. and is in the range of the values reported in the literature.

The collaborators found that the qualitative tests responded as described in the method if the directions were carefully followed. The Associate Referee and several of the collaborators observed that the qualitative color tests with sulfuric acid did not respond or that the purple color developed very slowly if too small an amount of mandelic acid was used; however, the odor of benzaldehyde was always noted. While the qualitative tests are none too sensitive, the Associate Referee believes that if they are used in conjunction with the melting point they will be of service in the identification of mandelic acid.

RECOMMENDATIONS[1]

It is recommended—

(1) That the qualitative tests be adopted as tentative.

(2) That the methods for the determination of mandelic acid in tablet and liquid preparations be adopted as tentative.

REPORT ON CHLOROFORM IN MIXTURES

By John R. Matchett (Treasury Department Laboratories, Washington, D. C.), *Associate Referee*

The present (tentative) method for the determination of chloroform in mixtures is due to Roberts and Murray[2] and to experimental studies carried on during 1930 by Matchett, *This Journal*, **14**, 360 (1931).

The method was published in *Methods of Analysis, A.O.A.C.*, 1935, 579.

During the present year the following criticisms and suggestions have been made by A.O.A.C. members and others:

1. Reagent **106(a)**, saturated KOH in methyl alcohol, might well be substituted for reagent **104(a)**, thus eliminating one reagent. The reagent proposed is necessary for hydrolysis of CCl_4.
2. The alkali solution at present specified is more concentrated than need be.
3. The term "citrate bottle" should be changed to "pressure bottle" and the words "fitted with a rubber gasket to provide a tight seal" added.
4. The directions should include the following words of caution with regard to the handling of pressure bottles: "Caution: Do not cool pressure bottle suddenly. It is best to allow it to cool in the H_2O in which it was boiled."
5. The amount of $CaCO_3$ added to the sample prior to distillation should be reduced from 1 gram to 0.1 gram.
6. Previous work by Associate Referee Matchett was inadequate, especially in that only three analyses indicating that a suitable recovery of $CHCl_3$ was possible were reported.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 58, 98 (1939).
[2] *Am. J. Pharm.*, **101**, 654 (1929).

DISCUSSION

The criticisms and suggestions are well taken. No objection can be offered to the adoption of the changes proposed under 1, 3, 4, and 5. It is probably true also that less concentrated alkali would serve equally well for the hydrolysis of the chloroform. The advantage of utilizing a single reagent for both chloroform and carbon tetrachloride, however, outweighs this consideration, especially since no particular disadvantage accrues from its use.

During the previous scrutiny of the method it developed, late in the season, that collaborative results, although reproducible as indicated by check analyses, were decidedly too low. Approximately 0.5 gram of chloroform per 100 cc. was found in samples to which approximately 0.9 gram per 100 cc. had been added. These findings led to the presumption that chloroform had been lost by evaporation, or otherwise, and experiments that corroborated this view were carried out.

It appeared advisable then to add known amounts of chloroform to individual samples immediately prior to distillation. This was done and the three recorded results obtained.

The recovery in these analyses, though not gratifying, appeared adequate for the purpose and no reason whatever was seen to indicate the results could not be repeated. It is true that no possible interfering substances, especially plant extractives, were present. The method, however, is prescribed, not for a specific type of sample but for chloroform in mixtures. Any mixture of unknown composition must be examined to insure the absence of interfering substances before being assayed by any method for any constituent, hence nothing appeared to be gained by adding extractive matter from any drug.

In view of the foregoing, the evident reproducibility of results, and the analyses recorded by Roberts and Murray, the Associate Referee recommended that the method be adopted as tentative. The recommendation was accepted and the subject closed at that time.

The editorial review of *Methods of Analysis*, requested by the General Referee on Drugs, made it desirable to further examine the method in the light of the criticisms and suggestions offered. To this end the analytical results summarized in Tables 1, 2, 3, and 4 are offered.

The following procedure was followed:

Table 1.—Samples containing a known weight of $CHCl_3$ (labeled c.p., containing approximately 0.5% alcohol) dissolved in 10 cc., of alcohol were pipetted into pressure bottles containing 25 cc. of saturated methyl alcoholic KOH and 60 cc. of alcohol. The bottles were sealed, and the analysis was carried on in the specified manner. Results indicated that the $CHCl_3$ used was 99.1% pure.

Table 2.—To 20 cc. portions of a sirup containing 625 grams of sugar and 40 grams of NH_4Cl per liter were added 65 cc. of alcohol and 10 cc. of an alcoholic solution containing a known amount of $CHCl_3$. The samples were distilled and analyzed as prescribed, alkaline reagent **106(a)** and 0.1 gram of $CaCO_3$ being used. Taking 99.1% of the weighed amount of $CHCl_3$ as the actual amount present, the average recovery in these analyses was 98.9%.

Table 3.—20 cc. samples of compound Sirup of White Pine, N.F. VI, stated to contain 3 minims of $CHCl_3$ per fluid ounce, were analyzed, and the results were recorded in Analyses 1, 2, and 3. To similar samples were added known amounts of $CHCl_3$ and the analysis was carried on in the same way at the same time. The results are shown in Analyses 4 and 5. The two analyses gave practically identical results with indicated recovery of 98.2% based on the $CHCl_3$ present (average of 1, 2, and 3) and 99.1% of the $CHCl_3$ weighed and added.

Table 4.—Two 200 cc. samples were prepared containing identical amounts of $CHCl_3$. In sample "A" the menstruum was the sirup referred to in Table 2, plus 10% alcohol; in sample "B" the menstruum was alcohol. Analytical samples were pipetted from each. The low recovery recorded under samples "A" indicates the necessity for exercise of extreme care in sampling.

TABLE 1.—*Samples not distilled*

	$CHCl_3$ ADDED	$CHCl_3$ FOUND	RECOVERY
	gram	*gram*	*per cent*
1	.1470	.1455	98.9
2	.1470	.1457	99.1
3	.1476	.1466	99.3
4	.1476	.1462	99.0
5	.1476	.1465	99.2
6	.1469	.1454	99.0
7	.1469	.1461	99.4
			Av. 99.1

TABLE 2.—*Samples distilled as directed from sirup containing 625 grams of sugar and 40 grams of NH_4Cl per liter*

	$CHCl_3$ ADDED	ACTUAL $CHCl_3$ ADDED*	$CHCl_3$ FOUND	RECOVERY
	gram	*gram*	*gram*	*per cent*
1	.1476	.1463	.1456	99.5
2	.1476	.1463	.1443	98.6
3	.1476	.1463	.1436	98.1
4	.1476	.1463	.1466	100.2
5	.1476	.1463	.1430	97.8
6	.1476	.1463	.1440	98.4
			Av.	98.8

* 99.1% of weight of added $CHCl_3$.

TABLE 3.—*Samples of compound sirup of white pine to Nos. 4 and 5 of which were added weighed amounts of $CHCl_3$ and the analyses conducted in manner prescribed*

	$CHCl_3$ PRESENT	$CHCl_3$ ADDED	$CHCl_3$ FOUND	RECOVERY
	gram	*gram*	*gram*	*per cent*
1		None	.0940	
2		None	.0933	
3		None	.0930	
4	.0934[1]	.0729[2]	.1650	98.2[3]
5	.0934[1]	.0729[2]	.1651	98.2[3]

[1] Average of Determinations 1, 2, and 3.
[2] 99.1% of $CHCl_3$ weighed.
[3] Based on difference between $CHCl_3$ found and average of $CHCl_3$ found in Analyses 1, 2, and 3.

TABLE 4.—*Samples A pipetted from sirup referred to in Table 2, plus 10% alcohol. Samples B pipetted from alcohol like those reported in Table 2. Analysis same as in Tables 2 and 3.*

	$CHCl_3$ ADDED	ACTUAL $CHCl_3$ ADDED*	$CHCl_3$ FOUND	RECOVERY
	gram	*gram*	*gram*	*per cent*
A	.1469	.1456	.1395	95.8
A	.1469	.1456	.1395	95.8
B	.1469	.1456	.1437	98.7
B	.1469	.1456	.1441	99.0

* 99.1% of weight of added $CHCl_3$.

OBSERVATIONS AND CONCLUSIONS

1. The use of reagent **106(a)** is satisfactory.
2. Use of 0.1 gram of calcium carbonate is satisfactory.
3. Carborundum chips are very useful to prevent "bumping" during distillation.
4. The method may be relied upon to show at least 98 per cent recovery of chloroform present in the sample.
5. As much as 5 per cent of the chloroform present may be lost on pipetting a sample of heavy sirup.
6. By use of special apparatus recovery might be increased and more concordant results obtained.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method be amended to reduce the calcium carbonate from 1.0 to 0.1 gram.

(2) That the method be amended to permit the use of carborundum chips to prevent bumping.

(3) That the term "citrate bottle" be deleted and the words "pressure bottle" be substituted.

(4) That the following caution be inserted in an appropriate place:

Caution: Do not cool the pressure bottle suddenly. It is best to allow it to cool in the water in which it was boiled.

(5) That the method (after amendments) be retained in its tentative status.

The analyses reported herein were made by Louis Benjamin, Joseph Levine, and G. F. Beyer, to whom the Associate Referee makes grateful acknowledgment.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 58 (1939).

CONTRIBUTED PAPERS

A RAPID METHOD FOR CHLORIDES IN TOMATO PRODUCTS

By L. M. BEACHAM (U. S. Food and Drug Administration, Washington, D. C.)

The present official method[1] for the determination of chlorides in tomato products requires drying on a steam bath and ashing, which makes the method slow and time-consuming. The tentative method[2] for chlorides in tomato juice, which it has been suggested might be used for other tomato products as well, requires filtering the product. Tomato products tend to clog the filter, and filtration is difficult. The following method has been found to obviate these difficulties and to give excellent results:

METHOD

Weigh 5 grams of the tomato material and transfer with 80% C_2H_5OH to a 100 cc. volumetric flask. Then add the C_2H_5OH to give a volume of approximately 50 cc. Shake well to get all the tomato material into suspension. Add 1 cc. of concentrated HNO_3 and by means of a pipet add 25 cc. of 0.1 *N* $AgNO_3$. Make to 100 cc. volume with alcohol, transfer to a centrifuge bottle, and centrifuge at 1800 r.p.m. for 5 minutes. Pipet 50 cc. of the supernatant liquid into a 300 cc. Erlenmeyer flask, add 2 cc. of a saturated solution of $FeNH_4(SO_4)_2$ and titrate to a permanent light brown color with 0.1 *N* NH_4CNS solution. Multiply the number of cc. of NH_4CNS used by 2 and subtract from 25. Multiply the difference by .005843 to obtain the weight of chlorides present expressed as grams of NaCl. Divide by 5 and multiply by 100 to calculate the percentage of salt present.

Analyses were made on a number of samples of tomato products by both the official method and the one described. Some typical results are given in Table 1.

TABLE 1.—*Typical results (NaCl) on tomato products*

PRODUCT	OFFICIAL METHOD	ALCOHOL EXTRACTION METHOD
	per cent	*per cent*
Paste	1.31	1.32
	1.32	1.32
Paste	2.14	2.13
	2.14	2.10
Paste	2.13	2.13
	2.13	2.13

Authentic samples of tomato paste and tomato juice were prepared and analyzed by the alcohol extraction method for salt naturally present. Known amounts of salt were also added to subdivisions of these authentic

[1] *Methods of Analysis, A.O.A.C.*, 1935, 500, 22.
[2] *This Journal*, 20, 78 (1937).

samples, and analyses were made by the alcohol extraction method to determine the total salt present. The results are given in Table 2.

TABLE 2.—*Salt naturally present and added*

PRODUCT	SALT ADDED	NATURAL SALT	TOTAL SALT BY CALCULATION	TOTAL SALT BY ALCOHOL EXTRACTION
	per cent	*per cent*	*per cent*	*per cent*
Paste	2.00	.314	2.314	2.320
				2.320
Juice	1.00	.042	1.042	1.047
				1.047

SUMMARY

A rapid method for determining the chlorides present in tomato products by means of alcoholic extraction and precipitation with silver nitrate is described. When compared with the present official method, the method gives equally accurate results, as it does when used on authentic samples having a known amount of chlorides added.

AN OBJECTIVE METHOD FOR MEASURING GRITTINESS IN CANNED PEARS

By L. M. BEACHAM (U. S. Food and Drug Administration, Washington, D. C.)

Grittiness is one of the varietal characteristics of canned pears. For example, pears of Kieffer variety have a high degree of grittiness, while Bartlett pears are relatively free from it. Investigation indicates that the grittiness is caused by the presence of grit cells approximately 0.02 inch or more in diameter. Pears containing only cells of smaller diameter, or even these smaller cells alone, may be chewed and swallowed without any impression of the presence of hard material.

The following method was developed for determining in canned pears the amount of grit cells retained on a 30-mesh screen (that is, of approximately 0.02 inch diameter):

METHOD

Drain the pears, with the "cups" down, for 2 minutes on an 8-mesh screen with wire diameter of .013 inch, using an 8-inch screen ("Standard Specifications for Sieves," U. S. Department of Commerce, National Bureau of Standards Bulletin of October 25, 1938) for containers of less than 3 pounds net weight, and a 12-inch screen for larger containers. Remove the pears to a food chopper and grind until homogeneous. Place 50 grams of the ground material in the cup of a malted milk stirrer (a Hamilton Beach Model 25 was used in these experiments), and add 200 cc. of water and 25 cc. of 50% NaOH solution. Bring the mixture to a boil and boil vigorously for 5 minutes; place under the stirrer and stir for 5 minutes; and filter

through a tared 30-mesh Monel metal wire screen ("Standard Specifications for Sieves," U. S. Department of Commerce, National Bureau of Standards Bulletin of October 25, 1938) fitted into a Büchner funnel. Wash with copious quantities of hot water until the grit cells are free of adhering pear material, and the wash water is free from visible cells. Dry screen and retained cells at 100° C. for 1 hour and weigh. Calculate the percentage of grit cells retained on the screen.

Samples of canned Bartlett, Kieffer, and Pineapple pears were examined. The results are listed in the table.

VARIETY	ORIGIN	TASTE	GRIT CELLS RETAINED
			per cent by weight
Bartlett	California	Smooth	.03 .02 .02
Bartlett	Oregon	Smooth	.03 .04
Bartlett	Michigan	Smooth	.04 .05 .05 .06
Bartlett	Michigan	Smooth	.02 .03
Bartlett	Oregon	Smooth	.01
Bartlett	Oregon	Smooth	.04 .06
Bartlett	New York	Smooth	.04 .04
Kieffer	Michigan	Gritty	.42 .40 .42
Kieffer	Michigan	Gritty	.48 .54
Kieffer	Michigan	Gritty	.36 .40
Pineapple	Mississippi	Gritty	.20 .19
Pineapple	Mississippi	Gritty	.27
Pineapple	Mississippi	Gritty	.31

SUMMARY

A method is described for separating and determining in canned pears grit cells retained on a 30-mesh screen.

A table shows the percentage by weight of such grit cells in samples of canned Bartlett, Kieffer, and Pineapple pears, and a comparison of the grit cells (per cent) with an organoleptic examination of such pears.

THE FREEZING POINT OF MILK

By LINCOLN M. LAMPERT (Dairy Service Laboratory, California State Department of Agriculture, Sacramento, Calif.)

In a paper, entitled "The Cryoscopy of Milk," Julius Hortvet[1] described the instrument now widely used for the measurement of the freezing point of milk. He reported the freezing-point depression of genuine milk to range from 0.534° to 0.562° C., with an average of 0.548° C. It would appear that the currently accepted value for the freezing-point depression for milk was obtained by rounding this figure to 0.55°. Bailey[2] presented data on collaborative work on milk from herds and normal individual cows as summarized in Table 1.

TABLE 1.—*Average freezing-point depression of authentic milk*
(From data presented by Bailey)[2]

MILK FROM INDIVIDUAL COWS	RANGE	AVERAGE
130 samples	0.523–0.580	0.546
Milk from 36 herds (Total of 299 cows)	0.529–0.557	0.545
Mixed milk from 2300 cows	—	0.541–0.540
Average of all values	—	0.544

Studies made in the Union of South Africa[3] gave results between 0.528° and 0.561° C., with an average of 0.541° C.

Stubbs and Elsdon[4] examined 1000 samples of milk, a few of which were obtained from individual cows, but mostly from herds. The freezing-point depressions, determined by the official A.O.A.C. method, ranged from 0.529° to 0.563°, the average being 0.544° C.

Data obtained by the Dairy Service Laboratory of the California State Department of Agriculture show that samples of pure milk often have a freezing-point depression well above 0.550° C. It was therefore deemed desirable to obtain freezing-point data on a few samples from different parts of the State. These were obtained by State Dairy Inspectors under conditions that insured that the samples were genuine and unadulter-

[1] *J. Ind. Eng. Chem.*, 13, 198 (1921).
[2] *This Journal*, 5, 484 (1922).
[3] *Analyst*, 62, 44 (1937).
[4] *Ibid.*, 59, 146 (1934)

ated. The samples were received in the laboratory in excellent condition. The A.O.A.C. method was carefully followed in obtaining the freezing-point depressions shown in Table 2. In practically every instance the

TABLE 2.—*Freezing-point depression of milk*

LAB. NO.	SOURCE	FAT	SOLIDS NOT FAT	FREEZING-POINT DEPRESSION	MILK FROM—
		per cent		°C.	
284	Sacramento	4.8	9.66	0.547	—
345	Salida	4.5	9.12	0.536	6 cows
346	Salida	4.78	9.80	0.537	6 cows
347	Salida	4.75	9.27	0.536	24 cows
348	Fresno	5.4	9.11	0.541	Guernsey cows
351	Fresno	5.9	9.68	0.541	Guernsey cows
354	Fresno	5.05	9.53	0.541	Guernsey cows
357	Fresno	5.35	9.32	0.541	Guernsey cows
384	Eureka	4.0	9.56	0.536	Mixed breeds
385	Eureka	5.35	9.29	0.534	Guernsey cows
434	Redding	3.4	8.50	0.537	—
436	Redding	4.4	8.60	0.536	—
448	Anderson	5.1	9.18	0.537	—
455	Sacramento	4.5	9.30	0.540	—
459	Yuba City	4.95	9.29	0.536	—
487	Artesia	3.6	8.59	0.532	210 Holstein, 75 Guernsey, 75 Jersey
488	Downey	4.5	8.77	0.533	240 Holstein, 180 Guernsey, 60 Jersey
489	Hynes	4.7	10.19	0.547	120 Holstein, 15 Guernsey, 15 Jersey
490	Hynes	4.9	8.74	0.537	100 Holstein, 25 Guernsey, 25 Jersey
541	Eureka	4.4	8.87	0.529	Mixed breeds
542	Eureka	5.4	9.11	0.529	Guernsey cows
615	Sacramento	5.4	9.00	0.530	—
616	Sacramento	4.5	9.88	0.530	—
1148	Lakeport	—	—	0.536	—
			Average	0.536	

results are the average of closely agreeing replicate determinations obtained independently by two individuals. The thermometer of the instrument was carefully standardized according to the A.O.A.C. procedure. As a further precaution, it was also calibrated by the U. S. Bureau of Standards, which furnished a certificate for the instrument showing calibration data practically identical with those obtained here.

A survey of the data obtained at this Laboratory and of other recently published data would indicate that the accepted average freezing-point depression of 0.550° is somewhat too high. This view-point has been ac-

cepted by the authorities of New South Wales,[1] who as a result of a survey of genuine samples found mixed milk occasionally to have a freezing-point depression of 0.535° C. They therefore considered it advisable to alter the freezing point standard for milk from −0.550° to −0.535° C. In Western Australia,[2] a similar action has been taken. There the standard for the freezing-point depression is set at not less than 0.540° C.

It is of interest at this time to point out that in Bailey's data on 130 samples of authentic milk from individual cows 29 (22.3 per cent) had a freezing-point depression of 0.540° C. or less, and that out of 37 samples of milk from herds, 22 (57.9 per cent) had a freezing-point depression of 0.540° C. or less. The data obtained by Hortvet, Bailey, and Stubbs and Elsdon are combined in Table 3.

TABLE 3.—*Freezing-point depressions and frequencies*

	FREEZING-POINT DEPRESSIONS	FREQUENCIES
	0.523	6
	0.529–.530	12
	0.531–.532	14
	0.533–.534	33
	0.535–.536	53
	0.537–.538	79
	0.539–.540	137
	0.541–.542	155
	0.543–.544	172
	0.545–.546	148
	0.547–.548	123
	0.549–.550	105
	0.551–.552	70
	0.553–.554	44
	0.555–.556	25
	0.557–.558	10
	0.559–.560	16
	0.561–.563	15
	0.564–.566	7
Average	0.544	Total 1224

These data were examined statistically, but the results are virtually the same as those obtained by mere inspection of the table. The distribution curve is normal and very symmetrical. The mean is 0.544, the median 0.543.

It will be noted in Table 3 that out of 1224 examinations, 661 (54 per cent) show a freezing-point depression of 0.540 or less, and 1037 (84.7 per cent) show a depression of 0.550 or less. Inasmuch as it has been proved that the season of the year or feed does not affect the freezing-point de-

[1] *Analyst*, 62, 610 (1937).
[2] *Ibid.*, 63, 890 (1938).

pression of milk from healthy cows, there is no reason to assume that these values are not characteristic of much of the milk produced. The writer believes it advisable, therefore, in cases where control samples are not available, that a freezing-point standard of 0.540° C. be accepted for pure milk, especially when the results are to be used for the detection and quantitative estimation of added water.

ACKNOWLEDGMENT

A number of the freezing-point determinations reported in this paper were made by John H. Brandon, of the Dairy Service Laboratory.

CONCLUSION

An examination of published data and of the new data presented indicates that 0.540° C. is a more desirable figure than 0.550° C. for the average freezing-point depression of pure milk.

ESTIMATION OF PSEUDO-CUMIDINE IN ACID DYES

By C. F. Jablonski (U. S. Food and Drug Administration, New York, N. Y.)

The Federal Food, Drug, and Cosmetic Act of 1938 makes mandatory the certification of all batches of coal-tar colors intended for use in foods, drugs, and cosmetics. Regulations governing the procedure for such certification and giving specifications for the purity of permitted coal-tar colors have been published by the Secretary of Agriculture.[1]

In these regulations there appears for the color FD&C Red No. 1, also known as Ponceau 3R, the specification: "Pseudo-cumidine, not more than 0.2 per cent." Pseudo-cumidine is one of the intermediates used in the manufacture of FD&C Red No. 1, and the following method is proposed for the estimation of small quantities of this intermediate, if present in uncombined form in the color. The method can also be used for the estimation of pseudo-cumidine in other acid dyes.

REAGENTS

(a) *Sodium hydroxide.*—Approximately 10%. Dissolve 10 grams of NaOH in 100 cc. of water.

(b) *Dilute sodium hydroxide.*—Approximately 0.125 *N.* Dissolve 1 gram of NaOH in 200 cc. of water.

(c) *Sulfuric acid.*—Approximately 1 *N.* Add 28 cc. of concentrated H_2SO_4 to 900 cc. of water. Cool, and dilute to 1 liter.

(d) *Sodium nitrite.*—10%. Dissolve 1 gram of $NaNO_2$ in 10 cc. of water.

(e) Schaeffer's salt.—1%. Dissolve 1 gram of beta-naphthol-6-sulfonate in 100 cc. of water.

(f) *Sodium carbonate.*—Approximately 2 *N.* Dissolve 110 grams of the anhydrous salt in 1 liter of water.

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[1] *Fed. Reg.*, **4**, 1935 (1939).

PROCEDURE

To 10.0 grams of the sample add 200 cc. of water and heat carefully on the steam bath until dissolved. When cool, transfer to a 250 cc. separatory funnel and add 10 cc. of the 10% NaOH. Add 40 cc. of $CHCl_3$ and shake vigorously for about 5 minutes. After separation, draw off lower layer into another funnel.

Re-extract the dye solution three times more with $CHCl_3$, using successively 30, 20, and 10 cc., and shaking vigorously each time. Discard the aqueous color solution. Wash the combined $CHCl_3$ extracts five times with 40 cc. each of 0.125 N NaOH and finally with 25 cc. portions of water until the washings are colorless (usually 3–4 washings).

Transfer the $CHCl_3$ extract to a 200 cc. beaker, add 75 cc. of 1 N H_2SO_4, mix, and evaporate carefully over a tepid water bath, using a current of air to aid volatilization. After removal of the $CHCl_3$ add sufficient water to make approximately 100 cc., place the beaker in an ice bath, and cool its contents to 3–5° C. Add 0.1 cc. of $NaNO_2$ solution and keep reaction mixture cold for about 1 hour.

In a 500 cc. Erlenmeyer flask place 5 cc. of Schaeffer's salt solution and 45 cc. of Na_2CO_3 solution. Pour the diazo mixture slowly into the alkaline Schaeffer's salt solution, mixing thoroughly. Place the Erlenmeyer flask with the red color solution on the steam bath, maintaining a temperature of about 70° C. for 1 hour for complete color development. (The intensity of the color is proportional to the pseudo-cumidine present.)

Cool the color solution and add 15 cc. of strong HCl. In each of three (or more if necessary) 250 cc. separatory funnels place 50 cc. of amyl alcohol. Transfer about 40 cc. of the color solution to the first funnel and extract. Draw off the lower (aqueous) layer into funnel No. 2 and repeat the extraction, transferring the aqueous layer to funnel No. 3 and so on, until all the red color is extracted. Discard the aqueous solution. Repeat in 40 cc. aliquots with the balance of the color solution, passing each aliquot from funnel to funnel in the same order and discarding them, as soon as the color is extracted.

Wash the amyl alcohol extracts successively with 40 cc. portions of water, passing washings from one funnel to another in reverse order to that used during the extraction until the water extract is colorless.

Dilute the amyl alcohol in each funnel with an equal volume of gasoline or ligroin and extract the coloring matter successively with 100 cc. portions of water, following the order used above for washing. Repeat the extractions until all the color is removed from each solvent mixture. Evaporate the combined aqueous solutions in a casserole over a live steam bath. Dissolve residue with 150 cc. of hot water, transfer to a 300 cc. Erlenmeyer flask, add 10.0 grams of Na bitartrate and titrate hot with standard $TiCl_3$ in presence of a current of CO_2.

1 cc. of 0.1 N $TiCl_3$ = 0.00338 gram of pseudo-cumidine.

A 10.0 gram sample of certified Ponceau 3R treated in the manner described gave 0.00067 gram or = 0.0067 per cent pseudo-cumidine. To another 10.0 gram aliquot of the same sample 0.010 gram of pseudo-cumidine was added before the treatment. The total amount of cumidine recovered was 0.00977 gram. Subtracting the blank (0.00067) the net recovery was 0.00911 gram, or 91.1 per cent.

EFFECT OF LIGHT ON ERYTHROSINE AND BROMO ACID

By O. L. EVENSON (Cosmetic Division, U. S. Food and Drug Administration, Washington, D. C.)

Erythrosine, the sodium salt of tetraiodofluorescein (Colour Index No. 773),[1] is one of the coal-tar colors that may be certified for use in coloring foods, drugs, and cosmetics in the United States. The color is listed[2] under the name FD&C Red No. 3 and to be certifiable it must meet the following specifications:

FD&C RED NO. 3

SPECIFICATIONS

Disodium salt of 9-o-carboxyphenyl-6-hydroxy-2, 4, 5, 7-tetraiodo-3-isoxanthone.

Volatile matter (at 135° C.), not more than 12.0%.

Water-insoluble matter, not more than 0.2%.

Ether extracts, not more than 0.1%.

Chlorides and sulfates of sodium, not more than 2.0%.

Sodium carbonate, not more than 0.5%.

Sodium iodide, not more than 0.4%.

Mixed oxides, not more than 1.0%.

Permitted range of organically combined iodine in pure dye, free from water of crystallization, 56.8–58.5%.

Pure dye (determined gravimetrically), not less than 85.0%.

Similarly bromo acid (tetrabromofluorescein) may be certified as D&C Red No. 21[2] for use in drugs and cosmetics, provided it meets the following specifications:

D&C RED NO. 21

SPECIFICATIONS

2, 4, 5, 7-tetrabromo-3, 6-fluorandiol.

Volatile matter (at 135° C.), not more than 6.0 %.

Insoluble matter (alkaline solution), not more than 1.0%.

Ether extracts (from alkaline solution), not more than 0.5%.

Chlorides and sulfates of sodium, not more than 2.0%.

Mixed oxides, not more than 1.0%.

Free bromine, not more than 0.02%.

Permitted range of organically combined bromine in pure dye, 47.5–51.5%.

Pure dye (determined gravimetrically), not less than 93.0%.

Several investigations have been made of the effect of light on dyes of the erythrosine-bromo acid type. Tappeiner and Raab[3] found that certain low forms of life were killed by exposure to light in the presence of eosin, the sodium salt of bromo acid. They attributed this to fluorescence and called substances exhibiting such effects, "Photodynamic." Dreyer[4] found that as silver bromide plates can be sensitized with erythrosine to the

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[1] Colour Index of the Society of Dyers and Colourists, England.
[2] *Fed. Reg.*, 4, 1936 (1939).
[3] *Münch med. Wochschr.*, 47, 5–7 (1900).
[4] *Proc. Royal Acad. Sci., Copenhagen*, No. 3, 393–97 (1903).

yellow and green rays of the spectrum, so can bacteria be sensitized to the same rays with this color. Neisser and Halberstadter[1] showed that bacteria were killed when sensitized with erythrosine and exposed to light. These investigators mentioned that iodine might be released from the erythrosine under the influence of light but concluded that therapeutic effects were due to the dye sensitizing the cells to the more deeply penetrating yellow and green rays rather than to the action of iodine. Jodlbaur and Tappeiner[2] postulated oxygen as the causative agent, since their experiments seemed to show that benzoic acid was produced from benzyl alcohol and that indigo was oxidized in the presence of erythrosine exposed to light. They also reported that exposed solutions of eosin and erythrosine developed acidity.

The following investigation of the influence of light on erythrosine and bromo acid was made incidental to determining the possibility of estimating these colors by a spectrophotometer. Certified colors were used and sodium iodide was determined by the procedure given in *Methods of Analysis*, of the Association of Official Agricultural Chemists, 1935, page 259. The extent of the fading of erythrosine was measured with a spectrophotometer.

From a freshly made aqueous solution of erythrosine (FD&C Red No. 3), containing 1 per cent of pure dye, a dilute aqueous solution containing 50 mg. of dye per liter was prepared; 20 cc. aliquots of this dilute solution (equal to 1 mg. of dye) were placed in each of four 100 cc. volumetric flasks. To two of these flasks 50 cc. portions of 95 per cent ethyl alcohol were added. Finally all four flasks were made to volume with water. These solutions were then exposed to direct sunlight for periods of 10 and 20 minutes. The Bunsen extinction coefficient ($E = -\log_{10}$ transmittancy) at 530 $m\mu$ was determined before and after exposure. The results are found in Table 1. Each of these results is the average of two closely agreeing determinations. It is evident from Table 1 that erythrosine fades rapidly in water solution, whereas in 47.5 per cent alcohol the rate of fading is much slower.

TABLE 1.—*Effect of exposure of erythrosine solutions to sunlight*

CONCENTRATION	SOLVENT	EXPOSURE	E* AT 530 $m\mu$	FADING
per cent		*minutes*		*per cent*
0.001	H_2O	0	.96	
0.001	H_2O	10	.77	20
0.001	H_2O	20	.62	35
0.001	47.5% alcohol	0	1.08	
0.001	47.5% alcohol	10	1.08	0
0.001	47.5% alcohol	20	1.05	3

* $E = -\log_{10}$ transmittancy. Layer depth = 1 cm.

[1] *Deut. med. Wochschr.*, 30, 265–69 (1904).
[2] *Deut. Archiv. klin. Med.*, 82, 520 (1904).

Experiments were then made on 1 per cent aqueous solutions of erythrosine, placed in 100 cc. volumetric flasks and exposed to the sun for periods of several months. At the end of the exposure an appreciable quantity of the characteristic color acid of erythrosine had precipitated. Sodium iodide was determined before and after exposure. Table 2 gives the results.

TABLE 2.—*Increase of sodium iodide in solutions of erythrosine after exposure to light*

NO.	SOLVENT	CONCENTRATION OF COLOR	TOTAL NaI IN SOLUTION	
			BEFORE EXPOSURE	AFTER EXPOSURE*
		per cent	*mg.*	*mg.*
1	H_2O	1	3	68
2	H_2O	1	3	125

* Time of exposure: No. 1—3½ months (Dec. 14, 1934 to April 2, 1935);
No. 2—6 months (May 1, 1934 to November 1, 1934).

Since the sodium iodide content increases in the exposed samples, it seems that the erythrosine molecule loses iodine under the influence of light. The presence of color acid in the exposed solutions further shows that the solutions developed acidity. The following reaction mechanism may explain this. Under the influence of light, iodine organically combined in the dye is split off. The iodine reacts with the slight excess of sodium carbonate that is always present in these colors. This results in the formation of carbon dioxide, which precipitates the color acid. A formation of hydriodic acid and iodic acid may be postulated as intermediary steps.

In the presence of erythrosine other dyes in aqueous solution also appear to be destroyed by the action of light. Table 3 shows the case of mixtures of erythrosine and sodium indigo disulfonate (also known to the trade as "indigotine" and certifiable as FD&C Blue No. 2). Mixtures of erythrosine and indigotine in 250 cc. volumetric flasks were exposed for about 3 hours to direct sunlight. The Bunsen extinction coefficients were determined at or near the two points of maximum absorption, 610 mμ for indigotine and 530 mμ for erythrosine. The percentage of indigotine destroyed, as computed from the reduction in the value of E, is shown in Table 3.

Table 3 indicates that the presence of as little erythrosine as 0.1 mg. per liter may have an appreciable effect on indigotine when the mixture is exposed to light. In mixture No. 4 the presence of 2 mg. of erythrosine caused a destruction of 14 mg., or 70 per cent of the indigotine present. Similar experiments on other water-soluble certifiable food colors showed that all except fast green (FD&C Green No. 3), brilliant blue (FD&C Blue No. 1), and tartrazine (FD&C Yellow No. 5) were destroyed to some

degree by direct sunlight in the presence and as a result of decomposition of erythrosine. The three colors mentioned appear to be quite stable, but the possibility remains that they, too, may be affected by a more prolonged exposure. The observation that certain coal-tar colors in the presence of erythrosine undergo decomposition and destruction in sunlight may be of practical significance, since erythrosine is used in food color mixtures.

TABLE 3.—*Effect of light on aqueous solutions of mixtures of indigotine and erythrosine*

TEST NO.	DYE	CONCENTRATION	E*			APPROXIMATE AMOUNT DESTROYED
			MEASURED AT WAVE LENGTH	BEFORE EXPOSURE	AFTER EXPOSURE†	
		mg./l.	*mμ*			*per cent*
1	Indigotine alone	20	610	0.88	0.88	0
			530	.18	.18	
2	Indigotine plus	20	610	.88	.83	6
	erythrosine	0.1	530	.18	.18	
3	Indigotine plus	20	610	.88	.52	40
	erythrosine	1	530	.28	.13	
4	Indigotine plus	20	610	.88	.28	70
	erythrosine	2	530	.38	.06	
5	Indigotine plus	20	610	.88	0	100
	erythrosine	20	530	2.20	.06	

* Layer depth 1 cm.
† Exposed 3 hours in 250 cc. volumetric flasks.

In the light of the experiments described it seems reasonable to assume that the fading of erythrosine is due to a release of halogen by the action of sunlight, resulting in the formation of a colorless compound. Experiments made in this laboratory also show that eosin (C.I. No. 768), fades rapidly on exposure to sunlight.

That bromo acid, the color acid of eosin, releases bromine in the presence of sunlight was shown in the following way. Dry bromo acid was placed in two 500 cc. Erlenmeyer flasks. A strip of starch iodide paper, held in place by a rubber stopper, was suspended in the neck of each flask as well as in the neck of a third flask, designated the blank and containing no bromo acid. The neck of each flask was covered with a stiff paper to protect the starch iodide paper from the direct rays of the sun. The blank and one of the flasks containing bromo acid were then placed in direct

sunlight for 10 hours. The remaining flask was placed in the dark. After exposure the starch iodide paper in the flask exposed to light and containing bromo acid turned blue when moistened with water, while the papers in the other flasks remained colorless. This experiment was repeated twice, and in both cases the results were the same. The release of free halogen from the dye as a result of the action of sunlight therefore seems to be definitely indicated. Since bromo acid is certifiable for use in drugs and cosmetics, the role of the released halogen should not be overlooked in considering the therapeutic, allergic, or antiseptic action of the color.

SUMMARY

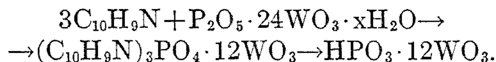
Aqueous solutions of erythrosine fade rapidly when exposed to direct sunlight, while the fading in 47.5 per cent alcohol is much slower. Iodine appears to be liberated from the erythrosine molecule and bromine from bromo acid by the action of light. Certain other dyes, in the presence of erythrosine, are destroyed, as a result of the action of light on the erythrosine present.

THE DETERMINATION OF QUINALDINE IN CERTAIN COAL-TAR COLORS

By W. H. King (Cosmetic Division,* U. S. Food and Drug Administration, Washington, D. C.)

Quinaldine (2-methyl quinoline) is an intermediate used in the manufacture of D&C Yellow No. 10 (Quinoline Yellow W.S.) and D&C Yellow No. 11 (Quinoline Yellow S.S.). Batches of these colors are acceptable for certification by the U. S. Department of Agriculture for use in drugs and cosmetics, provided they conform to certain specifications.[1] Among these specifications is one reading: "Quinaldine, not more than 0.2 per cent."

The following method is proposed for the determination of quinaldine in D&C Yellow Nos. 10 and 11. It is based on the separation of quinaldine from the colors by steam distillation, followed by extraction from the concentrated steam distillate and precipitation from acid solution as a salt of phosphotungstic acid, which salt may be weighed before and after incineration. The reactions involved are:



$$3C_{10}H_9N + P_2O_5 \cdot 24WO_3 \cdot xH_2O \rightarrow$$
$$\rightarrow (C_{10}H_9N)_3PO_4 \cdot 12WO_3 \rightarrow HPO_3 \cdot 12WO_3.$$

The test appears to be sensitive, 1–2 mg. of quinaldine in 100 cc. giving a distinct precipitate with phosphotungstic acid.

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* D. Dahle in charge.
[1] *Fed. Reg.*, 4, 1938 (1939).

METHOD

Place 25 grams of the color in a 2 liter flask equipped for steam distillation and having all glass connections. Add an excess of Na_2CO_3 (usually about 10 grams) in 50 cc. of hot water and steam distil. In the case of D&C Yellow No. 11 pass the steam through at a rather rapid rate to stir the insoluble dye during the distillation. Collect the distillate in a 1500 cc. beaker containing 1–2 cc. of concentrated HCl. Continue the distillation until all the quinaldine has come over (approximately 1000 cc.). Toward the end of the distillation drain the cooling water from the condenser and allow steam to pass through for several minutes. At the finish the liquid in the distilling flask must be alkaline to phenolphthalein (if not, repeat using more Na_2CO_3) and the distillate acid to congo red paper. Concentrate the distillate to approximately 100 cc. by boiling. If ammonium salts are present in the original sample, it is necesary to purify the quinaldine as directed under (*b*). Otherwise continue as directed under (*a*).

(*a*) Transfer the concentrated distillate to a 250 or 300 cc. beaker and add dropwise, with stirring, an excess of a 10 per cent filtered solution of phosphotungstic acid (about 1.5 cc. for each 10 mg. of quinaldine expected). Heat almost to boiling, let stand in a warm place until the precipitate has coagulated, and filter with suction on an ignited and weighed Gooch crucible. Dry to constant weight at 135° C. Cool in a desiccator and weigh as soon as cooled. The weight of dry salt multiplied by 0.131 gives the weight of quinaldine in the sample.

Ignite the contents of the crucible to constant weight at 500–550° C., cool as before, and weigh. The weight of the ash multiplied by 0.152 gives the weight of quinaldine in the sample.

(*b*) Transfer the concentrated distillate to a separatory funnel, neutralize with Na_2CO_3, adding 0.5–1.0 gram in excess, and extract with two 50 cc. portions of $CHCl_3$, washing each portion with 1–2 cc. of water in a second separatory funnel. Filter each portion through a small pledget of cotton into a 300 cc. tall-form beaker. Add to the combined $CHCl_3$ extracts 1 cc. of concentrated HCl and 100 cc. of water and boil off the $CHCl_3$. Proceed as directed under (*a*), beginning with the addition of the phosphotungstic acid.

From the weights W_1 and W_2 of $Q_3PO_4 \cdot 12WO_3$ and $HPO_3 \cdot 12WO_3$, respectively, calculate the molecular weight of quinaldine, Q. This gives a check on the identity of the amine. The formula,

$$Q = \frac{2864(W_1 - W_2)}{3W_2} - 5,$$

may be derived as follows:

If Q represents one molecule of quinaldine, the change taking place when W_1 grams of the salt, $Q_3PO_4 \cdot 12WO_3$, is ignited to give W_2 grams of the ash, $HPO_3 \cdot 12WO_3$, may be represented by—

$$Q_3PO_4 \cdot 12WO_3 - HPO_3 \cdot 12WO_3 = 3Q + O - H; \text{ and} \qquad (1)$$

$$W_1 - W_2 = \Delta W. \qquad (2)$$

If 1 mol of the salt is involved, 1 mol of the ash will be formed and the loss in weight on ignition, $(\Delta W)_m$, will be—

$$(\Delta W)_m = 3Q + O - H = 3Q + 16.0 - 1.0 = (3Q + 15) \text{ grams.} \qquad (3)$$

For X mols of salt, weighing W_1 grams, there would be formed X mols of ash, weighing W_2 grams, and the loss in weight, ΔW, would be—

$$\Delta W = X(\Delta W)_m = X(3Q + 15) \text{ grams.} \qquad (4)$$

Further, the relation between X, W_2, and the molecular weight of the ash, M.W., is—

$$\frac{\text{Actual weight of ash, } W_2}{\text{Molecular weight of ash, M.W.}} = \text{Number of mols, } X,$$

where M.W. is the molecular weight of $HPO_3 \cdot 12WO_3$, or 2864.0. This gives—

$$X = \frac{W_2}{2864}. \quad (5)$$

A combination of equations (2), (4), and (5) gives—

$$\Delta W = W_1 - W_2 = \frac{W_2(3Q + 15)}{2864}; \quad (6)$$

or

$$Q = \frac{2864(W_1 - W_2)}{3W_2} - 5. \quad (7)$$

When minute quantities of quinaldine are determined, i.e. $(W_1 - W_2)$ is small, equation (7) can, of course, only be depended upon to give a very rough estimate of Q.

DISCUSSION

Kahane and Kahane[1] have shown that with phosphotungstic acid certain simple organic bases form salts that have the general formula: $B_3(PO_4 \cdot 12WO_3)$. These compounds, upon ignition at dull red heat, are converted into the compound: $HPO_3 \cdot 12WO_3$.

According to these formulas, the factors for conversion to quinaldine (Q) would be—

$$0.130 \times Q_3(PO_4 \cdot 12WO_3) = Q;$$

and

$$0.150 \times HPO_3 \cdot 12WO_3 = Q.$$

The factors recommended in the method differ slightly, since they were determined experimentally, and take into consideration the solubility of quinaldine phosphotungstate. For the determination of these factors, Eastman's redistilled quinaldine, white label (216), was used in concentrations ranging from 5 to 200 mg. per 100 cc. Table 1 shows the results.

Several phosphotungstic acids are known,[2,3] the ratio, $P_2O_5:WO_3$, varying from 1:7 to 1:24. The compound most commonly referred to as "phosphotungstic acid" seems to be $P_2O_5 \cdot 24WO_3 \cdot xH_2O$. Barnes and Peters,[4] however, claim that commercial phosphotungstic acid is the "1:24 acid," containing some "1:18 acid."

[1] *Bull. Soc. Chem.*, 3, 621 (1936).
[2] Roscoe and Schorlemmer, Treatise on Chemistry, Vol. II, p. 1067. McMillan & Co., Ltd. (1907).
[3] Mellor, A comprehensive Treatise on Inorganic and Theoretical Chemistry, Vol. XI, p. 863. Longmans, Green & Co. (1931).
[4] *Biochem. J.*, 26, 2203 (1932).

TABLE 1.—*Factors obtained by precipitating various amounts of quinaldine with phosphotungstic acid*

QUINALDINE	FACTOR FOR DRY SALT	FACTOR FOR ASH
mg./100 cc.		
5	0.144*	0.169*
15	0.130	0.148
25	0.134	0.156
35	0.131	0.153
45	0.131	0.152
50	0.131	0.152
55	0.131	0.151
70	0.132	0.152
90	0.132	0.151
100	0.129	0.149
200	0.130	0.152
	Av. 0.131	Av. 0.152

* Not included in average.

In order to determine possible errors in the factors, due to variation in the composition of the phosphotungstic acid, experiments were made on eight commercial samples of the acid representing old and recent output of seven manufacturers; 50 mg. of quinaldine was used as a precipitant. The results are shown in Table 2.

TABLE 2.—*Factors obtained by precipitation of 50 mg. of quinaldine with various brands of phosphotungstic acid*

BRAND OF PHOSPHOTUNGSTIC ACID USED	FACTOR FOR DRY SALT	FACTOR FOR ASH
A	0.133	0.154
B (old)	0.131	0.152
B (new)	0.131	0.153
C	0.130	0.150
D	0.132	0.153
E	0.132	0.151
F	0.131	0.152
G	0.132	0.155
	Av. 0.131	Av. 0.152

While these results (Table 2) indicate a remarkable uniformity of commercial lots of phosphotungstic acid, it seems advisable that each analyst check his own batch of the acid before accepting the above-recommended factors.

Finally, known quantities of quinaldine were added to samples of D&C Yellow No. 10 and D&C Yellow No. 11, which samples, when examined by the proposed method, had not shown the presence of free quinaldine.

Recoveries ranged from 96 to 99 per cent, the main error being insufficient recoveries when less than 1000 cc. of distillate was collected. Incidentally, the fact that the samples prior to the addition of quinaldine failed to give positive results indicates that the isolation treatment does not decompose these colors.

DETERMINATION OF HYDROCYANIC ACID BY THE PICRIC ACID METHOD AND THE KWSZ PHOTOMETER

By J. T. SULLIVAN (U. S. Regional Pasture Research Laboratory, State College, Pa.*)

The picric acid test for hydrocyanic acid known as the Guignard test[1] is commonly used for qualitative, but less often for quantitative purposes. Some recent applications of the test for quantitative purposes have been made. Rogers and Frykholm[2] divided white clover plants into five groups according to the intensity of color of the test papers. Boyd, Aamodt, Bohstedt, and Truog[3] described a method that involves the heating of an alkaline picrate solution with the distillate of Sudan grass and a visual comparison of the color change with standards. In a private communication, F. T. Boyd of the Everglades Experiment Station, Florida, furnished more details of the method. B. W. Doak,[4] in a footnote to a recent paper, endorsed the Boyd procedure for white clover.

The procedure described here is an adaptation of the Boyd method to the determination of hydrocyanic acid in individual plants of white clover. Of all the plants studied 70 per cent contained .001 per cent or less of hydrocyanic acid on the fresh weight basis. Owing to the limited quantity of material available, a determination of less than .05 mg. of hydrocyanic acid was often necessary. Such small quantities could be more accurately determined by the KWSZ photometer than by visual comparison with standards.

THE METHOD

REAGENTS

(a) *Alkaline picrate solution.*—Dissolve 25 grams of anhydrous Na_2CO_3 and 5 grams (corrected for moisture content) of picric acid in 1000 cc. of water.

(b) *Potassium hydroxide.*—2% solution.

(c) *Toluene.*

(d) *Copper sulfate.*—10 grams of $CuSO_4 \cdot 5H_2O$ and 1 drop of H_2SO_4 dissolved in water and diluted to 100 cc.

(e) *Potassium cyanide.*

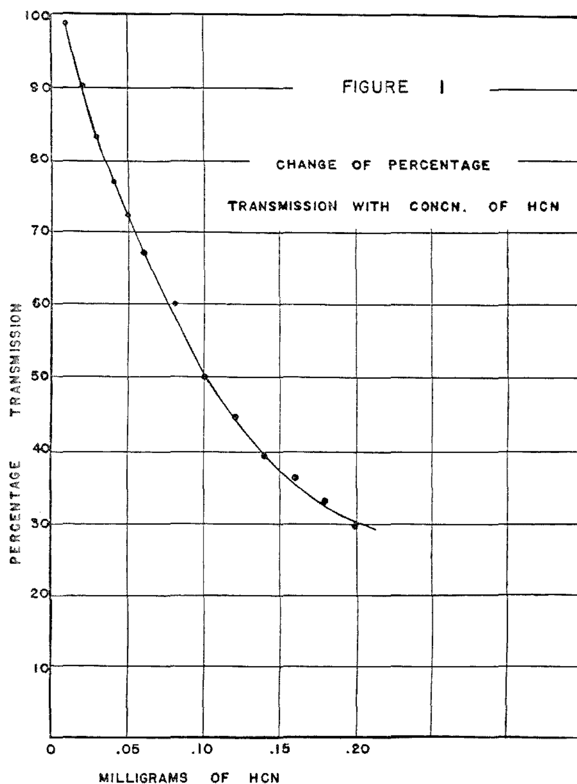
---

* A contribution from the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Northeastern States.

[1] *Compt. rend.*, 142, 545–53 (1906).
[2] *J. Agr. Res.*, 55, 533–37 (1937).
[3] *J. Am. Soc. Agron.*, 30, 569–82 (1938).
[4] *New Zealand J. Sci. Tech.*, 10, 3A, 163–66 (1938).

PROCEDURE

Place 10 grams of fresh white clover leaves in a 500 cc. short-necked Kjeldahl flask and add 5 cc. of toluene. Close the flask tightly with a rubber stopper, and allow to stand several days at room temperature. Remove the stopper, immediately make connection to a distilling apparatus, and steam distil from the same Kjeldahl flask. Distil 80–90 cc., catching the distillate in a 100 cc. beaker containing 5 cc. of the KOH. During the first half of the distillation have the tip of the condenser dipping below the surface of the liquid in the beaker. Transfer the distillate into a 100 cc. volumetric flask and dilute to the mark with water, mean-



while decanting and discarding the supernatant toluene. Pipet 20 cc. or less of the distillate and 10 cc. of the alkaline picrate solution into a 25×150 mm. test tube. (If less than 20 cc. of distillate is used, add water to a total volume of 30 cc.) Plug the test tube loosely with cotton and stand it upright in boiling water for exactly 5 minutes; remove, and cool to room temperature. With each set of determinations prepare a blank by heating 10 cc. of the alkaline picrate solution with 20 cc. of water or with the same volume of 0.1% KOH solution. Without diluting or rinsing, pour the entire contents of a test tube into one of the two absorption cells of the photometer. Balance the instrument against the blank, using the $CuSO_4$ solution as a light filter, and read the transmission of the unknown in the other absorption cell. From the curve prepared from standards, convert the percentage transmission into weight of HCN.

STANDARDIZATION

The standardization curve was prepared as follows: 1.015 grams of KCN (Baker's analyzed, assay 95.0%) was dissolved in 1 liter of water to give a solution containing 0.4 mg. of HCN per cc. Its strength was checked by alkaline titration against 0.02 N $AgNO_3$. A dilution was then prepared with 9 parts of water to give a solution containing 0.04 mg. of HCN per cc. A series of standards was made from this solution by diluting to 100 cc. aliquots varying from 1.25 to 25.0 cc.; 20 cc. fractions of these standards, containing 0.01–0.20 mg. of HCN, were heated as before with the alkaline picrate solution. The percentage transmissions were determined and plotted against the amount of HCN present. The curve shown in Figure 1 was drawn freely through the points obtained.

DISCUSSION

The conditions under which hydrocyanic acid is liberated from its parent glucoside and distilled need more exact definition. Immediate distillation of a sample with chloroform gave in some cases as high results as could be obtained with that particular sample, but in most cases higher results were obtained if an autolysis period of 24–48 hours intervened before the distillation. The same was true with toluene, but the latter, being lighter than water, had the advantage of not interfering with the making of the distillate to volume. An autolysis of several days without any preservative, or freezing and thawing followed by distillation with or without preservation, gave variable results. Autolysis for one or two days at room temperature with toluene always gave as high or a higher yield of hydrocyanic acid than any other treatment of a duplicate sample. Grinding of the fresh sample was omitted, but it would undoubtedly influence the time necessary for autolysis. The addition of water to the sample did not appear necessary, and its omission hastened the steam distillation. Immediate heating of the distillate with the alkaline picrate is advisable, but the reading of the results in the photometer may be delayed a day or two.

Because of the deep color of the picric acid reagent little difference in light transmission between samples containing low and high amounts of hydrocyanic acid was shown when the photometer was balanced against water, either without a filter or when a 450 or a 650 mμ glass filter was used. The curve found most useful (Figure 1) was obtained by using a copper sulfate solution in the water bottles of the photometer as the only light filter and balancing the instrument against a blank determination containing the alkaline picrate reagent.

The range of variation of percentage transmission in triplicate determinations of the same distillate averaged 0.4 over the whole curve; that of duplicate distillations was greater, being about 3.0 when 0.1 mg. of hydrocyanic acid was present. There is a greater sensitivity at lower concentrations. It is not advisable to use aliquots containing more than 0.2 mg. of hydrocyanic acid.

In order to study the agreement with the alkaline titration method[1] it was necessary to use larger samples than are usually available and to use large dilutions for the picric acid test. The results, which follow, average 8 per cent higher than those by the titration method.

	PICRIC ACID METHOD			TITRATION		
	ALIQUOT OF DISTILLATE	ACTUAL HCN DETERMINED	TOTAL HCN	ALIQUOT OF DISTILLATE	ACTUAL HCN DETERMINED	TOTAL HCN
		mg.	*mg.*		*mg.*	*mg.*
1	1/20	.140	2.8	4/5	2.00	2.50
2	1/100	.055	5.5	3/4	3.75	5.00
3	1/100	.059	5.9	4/5	4.27	5.34
4	1/100	.0325	3.25	4/5	2.61	3.27

SUMMARY

A procedure is proposed for the determination of small quantities of hydrocyanic acid in individual white clover plants. It utilizes the KWSZ photometer for the measurement of the color change in the alkaline picrate—hydrocyanic acid reaction. Hydrocyanic acid may be measured in amounts of 0.01–0.20 mg. The various steps of the procedure, especially the conditions of autolysis and distillation, need further study, particularly if the method is applied to other plants than white clover.

DETERMINATION OF 1,4 DIHYDROXY-ANTHRAQUINONE IN D&C GREEN NO. 5

(Alizarine Cyanine Green F)

By G. R. Clark (Cosmetic Division,* U. S. Food and Drug Administration, Washington, D. C.)

The regulations[2] governing listing and certification of coal-tar colors under the Federal Food, Drug, and Cosmetic Act of 1938 limit the amount of the intermediate 1,4 dihydroxy-anthraquinone permitted in the color D&C Green No. 5 to not more than 0.2 per cent.

The colorimetric method presented here is suggested for the determination of 1,4 dihydroxy-anthraquinone. The intermediate is separated by continuous extraction with diethyl ether from a slightly acid solution of D&C Green No. 5 and is in turn removed from the ether by a 5 per cent sodium hydroxide solution, with which reagent it forms a characteristic reddish-blue color.

Eastman's 1,4 dihydroxy-anthraquinone, technical grade, was used for these experiments. The material was purified by precipitation with hydrochloric acid from an alkaline solution, followed by three crystallizations of the precipitate from alcohol.

[1] *This Journal*, **19**, 94 (1936).
* D. Dahle in charge.
[2] *Fed. Reg.*, **4**, 1935 (1939).

PROPOSED METHOD

REAGENT

Sodium hydroxide.—5%. Dissolve 5 grams of NaOH in 100 cc. of water.

1,4 *Dihydroxy-anthraquinone*.—A freshly prepared, solution containing 0.1 gram of 1,4 dihydroxy anthraquinone dissolved in 100 cc. of a 5% NaOH solution.

DETERMINATION

Dissolve 2 grams of sample in 20 cc. of the 5% NaOH solution, dilute to 100 cc. transfer to a continuous extractor, and add sufficient concentrated HCl to neutralize the NaOH and give 2–3 drops (not more) in excess. Make to a volume suitable for the extractor and extract with ether, testing 50 cc. portions of the extract from time to time by shaking in a small separatory funnel with a few cc. of the 5% NaOH reagent until the characteristic reddish-blue color fails to develop (complete extraction). Save the ether and also the NaOH solution used in testing for complete extraction. (four hours was found to be more than sufficient to extract 6 mg. from 300 cc. of sample solution.)

Evaporate the ether extracts, including the portions tested for complete extraction, to about 75 cc.; transfer to a separatory funnel, and extract with 25 cc. portions of the 5% NaOH reagent until no more color is extracted. (Some D&C Green No. 5 may remain in the ether layer.) Combine the NaOH extracts with the NaOH previously used in testing for complete ether extraction, and shake in a separatory funnel with 10–15 cc. portions of ether until all D&C Green No. 5 is removed.

Make the volume of the combined NaOH extracts to 250 cc. with the 5% NaOH reagent, mix, and pipet 100 cc. into a suitable comparison tube. Place about 98 cc. of the 5% NaOH reagent in another comparison tube, which serves as the standard. From a 10 cc. buret, graduated in 0.05 cc., add 0.1% solution of 1,4 dihydroxy-anthraquinone to the standard tube until the color of the standard matches the unknown. For the final color comparison dilute both solutions to the *same* volume with the 5% NaOH reagent. Calculate the amount of 1,4 dihydroxy-anthraquinone present as follows:

$$\frac{\text{cc. standard used} \times 0.25}{\text{sample weight}} = \%\text{ of 1,4 dihydroxy-anthraquinone present.}$$

DISCUSSION

A 2 gram sample of D&C Green No. 5 contained sufficient 1,4 dihydroxy-anthraquinone to require 0.8 cc. of the standard, equivalent to 2 mg., or 0.1 per cent; 4.5 mg. of 1,4 dihydroxy-anthraquinone was added to 2 grams of this dye, and the recovery was determined. The amount of standard required to match the color was 2.5 cc. This is equivalent to 6.25 mg., which, less the 2 mg. originally present, indicates a recovery of 4.25 mg. of the 4.5 mg. added, or 94 per cent.

BOOK REVIEW

Standard Chemical and Technical Dictionary. By H. BENNETT. Chemical Publishing Co., Inc., New York, N. Y. 1939. 638 pp. Price $10.00.

In these days of specialized scientific research and writing it would seem that it is not possible to have too many sources of information to assist the searcher in acquiring the necessary knowledge to treat his subject efficiently and thoroughly.

Therefore this publication will be welcomed by all scientists working in the field covered. Its author asserts that it is "A condensed technical work book for students, writers, technicians, engineers, scientists, and all others who need assistance in keeping up with the many new chemical, physical, mathematical, and technical words and expressions."

The work seems to be well done and the entries quite adequate. Only continued practical use of a dictionary, however, can prove its worth. If on first trial the user finds that his special subjects are treated to his satisfaction, he considers it a good book; if, on the other hand, he expects the unusual and does not find it, he disapproves, or even condemns a publication before giving it a fair trial. Therefore the writer can not be too critical when she did not find such entries as "rose bengale," 'desoxycholic acid," and "Reinecke salt," and considers that not enough informaion was given for the word "esculin."—MARIAN E. LAPP.

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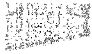

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