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OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

Vol. 30 FEBRUARY, 1947 No. 1

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ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS—OFFICERS, 1947

President, J. O. CLARKE, U. S. Food and Drug Administration, Chicago 7, Ill.
Vice-President, G. H. MARSH, Director Chemistry Division, Montgomery, Ala.
Secretary-Treasurer Emeritus, W. W. SKINNER, Kensington, Md.
Secretary-Treasurer, HENRY A. LEPPER, U. S. Food and Drug Administration, Washington 25, D. C.
Additional Members of Executive Committee, L. S. WALKER, Burlington, Vt.; W. A. QUEEN, Washington, D. C.; H. A. HALVORSON, St. Paul, Minn., and W. H. Ross, Washington, D. C.
Committee on Recommendations of Referees, W. F. REINDOLLAR (Bureau of Chemistry, State Department of Health, Baltimore 18, Md.), *Chairman*.
Subcommittee A: G. E. GRATTAN (1948), Department of Agriculture, Ottawa; H. A. HALVORSON (1950), and E. L. GRIFFIN (1952).
Subcommittee B: HARRY J. FISHER (1948), Conn. Agricultural Experiment Station, New Haven, Conn.; G. R. CLARK (1950), and F. H. WILEY (1952).
Subcommittee C: C. S. LADD (1948); JOSEPH CALLAWAY (1950), and P. A. CLIFFORD (1952).
Subcommittee D: C. S. FERGUSON (1948), State Department of Public Health, Boston, Mass.; KENNETH L. MILSTEAD (1950), and J. WALTER SALE (1952).

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Manuscripts should be typewritten, double-spaced, carefully revised before submission, and the original and one carbon submitted to the Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D. C.

Reprints will be furnished at cost to contributors of articles printed in *The Journal*. A table showing cost, with an order slip, is sent by the printer with proof.

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METHODS OF ANALYSIS, 6th edition, 1945

The list price of the new edition will be $6.25, domestic postpaid.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS—OFFICERS, 1947

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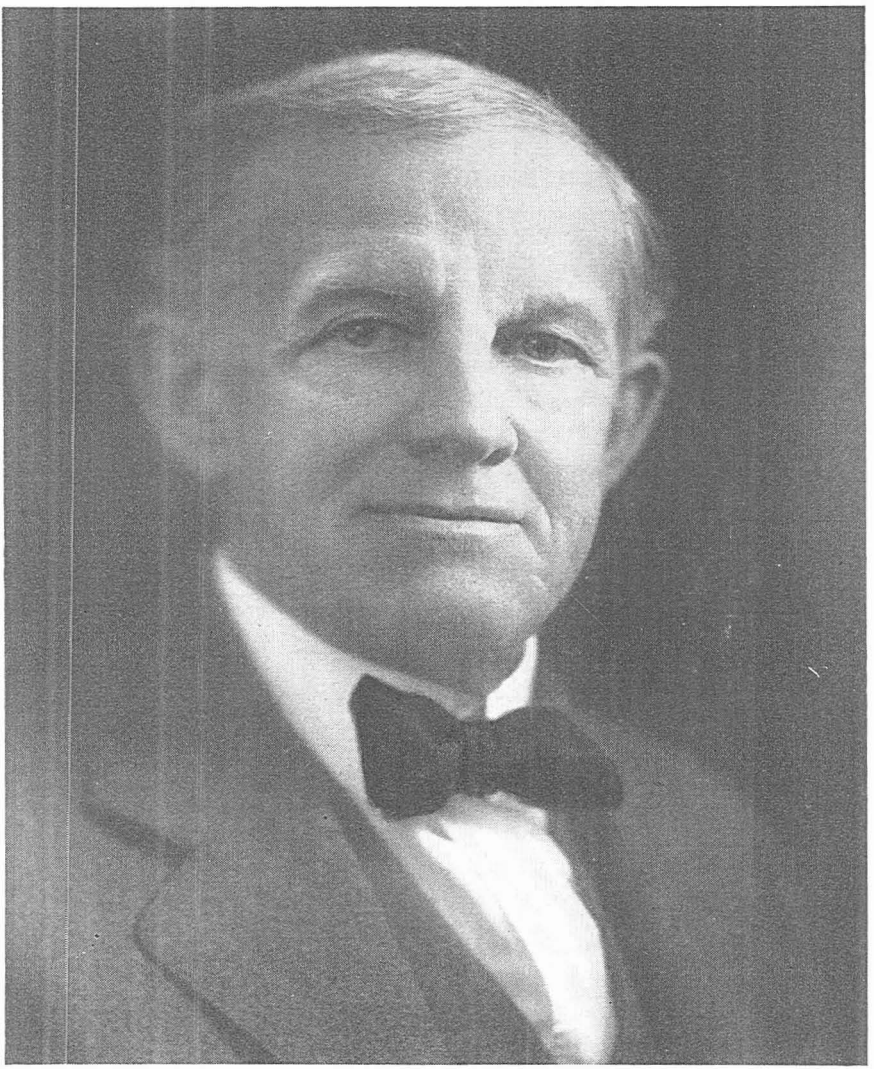

ANDREW LINCOLN WINTON, 1864–1946

ANDREW LINCOLN WINTON

One of the most remarkable coincidences in the history of the A.O.A.C. occurred during the week of its last annual meeting, when two of its pioneer members passed away within three days of the adjournment at almost exactly the same age of 82 years. These were Dr. Andrew L. Winton, who died on October 17, and Dr. Walker Bowman, who died on October 19. It would be interesting to trace the courses of their parallel lives—of Winton as an early assistant at the Connecticut Agricultural Experiment Station at New Haven and of Bowman as the first chemist of the Virginia Agricultural Experiment Station. Bowman's connection with Agricultural Chemistry, however, was very brief as he soon abandoned experiment station work to follow the profession of Chemical Engineer. The present sketch, therefore, is devoted to tracing the important influence of Winton upon the development and work of our Association.

Andrew Lincoln Winton was born at Westport, Connecticut, January 26, 1864, the son of Andrew Leavenworth Winton and Mary Esther (Gorham) Winton. After attending the elementary and high school at Bridgeport, Connecticut, he entered the Sheffield Scientific School of Yale University, from which he was graduated with the degree of Ph.B. in 1884. It was at "Shef" that he came under the instruction of Samuel William Johnson, professor of theoretical and agricultural chemistry, Director of the Connecticut Agricultural Experiment Station, and a pioneer leader of the agricultural experiment station work in the United States. Winton was never weary of expressing his indebtedness to the instruction and personal influence of Professor Johnson.

Immediately after graduation from "Shef" Winton began work as a member of the staff of the Connecticut Agricultural Experiment Station on September 1, 1884, which was only eight days before the date of the founding of the A.O.A.C. Thus he was eligible to membership in our Association on the date of its formation. Of others entitled to this honor, H. A. Huston is probably the only one now surviving.

During Winton's first three years at the Connecticut Experiment Station, in addition to analytical chemical work, he carried out a self-directed course in vegetable histology, using Bower and Vines text-book and Moeller's treatises for references. This was the beginning of his work in a special field that engaged his attention up to the time of his death.

Winton's first attendance at a meeting of our Association was at the eighth Annual Convention in 1891, at which time he presented a paper, "On the use of Sodium Chloride in the Lindo-Gladding Method of Determining Potash." From this time until 1911, he attended all the meetings of the Association except the ones in 1899 and 1904, when he was studying in Europe, and the one at Denver in 1909.

At the 1892 meeting, Winton presented the results of a study of Water in Cheese and, as reporter on Dairy Products, read additional papers in this field in 1893 and 1894. In the latter year he was appointed associate reporter on Potash and a member of the Abstract Committee. In 1895 he gave the report on Potash with H. J. Wheeler as co-author and was then made full reporter on this subject. Additional reports on Potash were presented in 1896 and, with C. H. Jones as collaborator, in 1897. In his extensive work for the Association as Reporter on Potash, Winton showed that the factor for potash, which had been adopted in both Europe and America, was based on Fresenius' faulty method of obtaining the double platinum salt. Winton's active participation in the work and discussions of the Association led to his election as Vice President for 1897 and as President for 1898.

Winton's presidential address is noteworthy for the announcement of certain fundamental principles that were influential in shaping the future policies of the Association. There was a difference of opinion at this time as to whether the referee work was a test of the proficiency of the analysts or a means for the improvement of analytical methods. Winton held steadfastly to the opinion that this collaboration was solely for the trying-out of methods and not for checking the analytical ability of chemists. In his address, he also emphasized the need of appointing a referee on insecticides and called attention to the importance of establishing commodity standards. He laid down the principle that "The adoption of standards of purity should go hand-in-hand with the adoption of methods of examination, as the proper interpretation of results is as essential as the accuracy of their determination." (*U.S.D.A.*, Bul. 56, pp. 9–14.)

Winton frequently asserted that in the development of regulatory control it was somewhat anomalous that plant foods or fertilizers should be investigated first, animal foods second, and human foods last of all. He was a most enthusiastic supporter of the pure food movement inaugurated by Dr. Wiley. Under the Association's program on Liquor and Food Adulteration, in 1900 he was appointed Associate Referee on Spices, Condiments, and Baking Powder. He had already, for a number of years, investigated the composition of more than a hundred samples of spices of all grades and qualities supplied by importers in New York and Boston, in which research his methods of microscopic examination were most useful.

For the purpose of perfecting himself in microscopic technique, he had spent six month in 1899 at the University of Graz, working under the direction of Professor Josef Moeller, the world's leading authority on this subject. This course with Professor Moeller was of so great benefit that Winton made additional collaborative microscopic investigations with him in 1902 and 1904. The results of their collaborative studies appeared in articles in the *Zeitschrift für Untersuchung der Nahrungs-und Genussmittel*, in the second enlarged edition of Moeller's *Mikroskopie der Nahrungs- und Genussmittel*, Berlin, 1905, and in Winton's *The Microscopy of Vegetable Foods*, New York, 1906.

Winton became more closely identified with the new Federal pure food movement when he resigned his position at the Connecticut Agricultural Experiment Station to become Chief of the Chicago Food and Drug Laboratory of the United States Bureau of Chemistry in 1907. Following this appointment Winton's work for the Association related to the detection of the adulteration of flavoring extracts, to the development of tests for bleached flour, and to other important problems relating to food control. Since 1905, he had been chairman of Committee C and presented the recommendations of its referees at each meeting until 1912, when the report was read by Trowbridge.

There were much closer bonds of good fellowship among the members of the Association during the long, inspiring secretaryship of Dr. Wiley than was afterward the case and Winton's magnetic personality was a strong factor in achieving this result. His ready wit, gift of repartee, and fund of amusing stories made him the central figure of many a merry group. A typical example of his occasional pranks, which created much amusement at the time, was his facetious dramatization at a luncheon with a small part of friends, of a session of the Food and Drug Inspection Board, at which he impersonated Dr. Wiley, with Doolittle and Mitchell acting as the two other members of the farce. His boon companionship once caused John Street to remark that it was worth a trip to an A.O.A.C. Convention just for the opportunity of meeting Winton.

Winton exercised indirectly an important influence upon the food control movement through his successive editions of Leach's *Food Inspection and Analysis*.

These were begun with the second edition of this work in 1909 when, to help out his friend Leach, who was ill, Winton voluntarily assumed the task of revision without any thought of remuneration. To quote from the preface to this edition, "Indeed, the work of revision has been due to the untiring energy of Dr. A. L. Winton, . . . who out of a busy life has taken entire charge of the details of the task, supplying most of the new material, as well as introducing much that is original as the result of his ripe experience." After the death of Leach, in 1910, Winton continued these revisions through the fourth edition, 1920, when his participation ceased because he had become convinced that changed conditions demanded an entirely new book on a different plan.

The resignation of Dr. Wiley as Chief of the Bureau of Chemistry in 1912 was followed by Winton's own withdrawal from the Government service in 1914. He had meanwhile been married in 1911 to Dr. Kate G. Barber, assistant chief of the Microanalytical Laboratory of the United States Bureau of Chemistry, whose collaboration was to be of so great value to Winton as microscopist and author in his future professional activities.

He purchased an old colonial house at Wilton, Connecticut, which he gradually converted into a repository of interesting antique furniture of Connecticut origin, many pieces of which he had skillfully restored in his own workshop. These he enjoyed describing to the many friends whom he and Mrs. Winton so delightfully entertained. It was here that they established laboratories for food research and the investigations incident to his work as consulting expert. It was here, also, that the technical contributions, for which the Wintons became best known, were written (see Addendum).

In 1936 Winton was invited by Secretary Skinner to give the sixth Wiley Memorial Address before the fifty-second convention of the A.O.A.C. Winton chose as his subject "Structure as an Approach to Food Chemistry" and took advantage of this opportunity to present his ideas upon a field of research in which he had specialized for over fifty years. He began his address by stating: "This lecture is an answer to an imaginary charge of chemical heresy. The accused pleads guilty, freely admitting that for the first quarter century of his active career he studied the sacred literature of that cult of botanists known as histology, albeit with the purpose of thereby aiding in the repression of fraud. He further admits that during the second quarter century, being thoroughly imbued with the spirit of liberalism, he and another [Mrs. Winton], both relieved of official service, have departed still further from orthodoxy, advocating that every food chemist be christened at the Malpighian font before he is cloistered in research." (*Journal A.O.A.C.*, vol. 20, p. 24, 1937.)

In the development of his lecture, however, when he stresses the great opportunities for the use of chemical stains in histological research, it becomes evident that Winton's departure from chemical orthodoxy was not actually so great as he so picturesquely leads us to suppose. This is revealed very strikingly when later, indulging in a characteristic, daring flight of imagination he states "With our present technique we see only the most abundant constituents of the cereals, but I am confident that by color reactions and other microchemical tests we shall eventually be able to locate even the vitamins." (*Loc. cit.*, p. 29.)

Lack of space prevents giving an account of the many important court cases in which Winton testified as food expert. We must also pass over with bare mention the extensive global travels of the Wintons during which they had opportunities of exchanging ideas with foreign scientsts and of collecting a great variety of rare plant food specimens for their laboratory investigations.

Winton's interest in the work of our Association continued unabated to the very

last. This is indicated in the last letter to his friend, H. A. Huston, written only nine days before his death, in which he says: "For months I have been looking ahead to the A.O.A.C. meeting as a kind of grand revival, but up to a day or two I have felt that my chances were fast dwindling with a heart that is heartless as to my interests. Somehow, today I have a faint hope that I may get there by auto, stopping this side of Washington and running into the meeting for an hour or two which is all my heart will stand. . . . I want the pleasure once more of telling the stories of Lawes, Gilbert, Maerker, and Liebig learned through my dear Professor S. W. Johnson, also of many others on this side—Babcock with his fidgety laugh, Jordan, Wheeler, and many others of whom we are proud. The greatest disappointment, if I don't get there, is not seeing you."

It is regretted by every member of our Association that this last wish of one of our oldest and most influential co-workers was not to be realized. His heart, badly weakened by a long illness the previous year, had been brought to the breaking point by over-exertion and his survival was only a matter of days. There are few careers in the history of the A.O.A.C. that display so many varied points of interest as that of Winton, as regard originality and accomplishments. For a long time to come a study of his achievements will be an inspiration to the members of our Association.

ADDENDUM

Publications of Andrew L. Winton and Kate Barber Winton:

HANAUSEK, T. F.: *The Microscopy of Technical Products*, 1907. Translated by Andrew L. Winton with the collaboration of Kate G. Barber.

WINTON, A. L.: *The Microscopy of Vegetable Foods*, Second Edition, 1916, with the collaboration of Dr. Josef Moeller and Kate Barber Winton.

WINTON, A. L.: *A Course in Food Analysis*, 1917.

WINTON, ANDREW L., AND KATE BARBER WINTON: *The Structure and Composition of Foods*, Vol. I, Cereals, Nuts, Oil Seeds, 1932; Vol. II, Vegetables, Legumes, Fruits, 1935; Vol. III, Milk, Eggs, Meat, Fish, 1937; Vol. IV, Sugar, Cocoa, Coffee, Tea, Spices, Leaven, 1939.

————: *The Analysis of Foods*, 1945.

C. A. BROWNE

PROCEEDINGS OF THE SIXTIETH ANNUAL MEETING OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1946

The sixtieth annual meeting of the Association of Official Agricultural Chemists was held at the Shoreham Hotel, Washington, D. C., October 14, 15, and 16, 1946.

The meeting was called to order by the President, William H. Ross, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, Beltsville, Md., on the morning of October 14, at 10:00 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING NOVEMBER 1947

President

J. O. CLARKE, U. S. Food and Drug Administration, Chicago 7, Ill.

Vice-President

G. H. MARSH, Director, Chemistry Division, Montgomery, Ala.

Secretary-Treasurer Emeritus

W. W. SKINNER, Kensington, Md.

Secretary-Treasurer

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Additional Members of the Executive Committee

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W. A. QUEEN, Washington, D. C.
H. A. HALVORSON, St. Paul, Minn.
W. H. ROSS, Washington, D. C.

PERMANENT COMMITTEES

Recommendations of Referees

(Figures in parentheses refer to year in which appointment expires.)

W. F. REINDOLLAR (Bureau of Chemistry, State Department of Health, Baltimore 18, Md.), *Chairman*

SUBCOMMITTEE A: G. E. GRATTAN (1948) (Department of Agriculture, 79 Sussex St., Ottawa, Can.), *Chairman;* H. A. HALVORSON (1950), and E. L. GRIFFIN (1952).

- Feeding stuffs
  - Mineral mixed feed (calcium and iodine)
  - Lactose in mixed feeds
  - Fat in fish meal
  - Adulteration of condensed milk products
  - Fat in cooked animal feeds containing cereals
  - Crude fat or ether extract
  - Activity of yeast
  - Microscopic examination
  - Fluorine
  - Mineral constituents of mixed feeds
  - Crude fiber
  - Protein evaluation in fish and animal products
  - Crude protein (catalysis)
  - Hydrocyanic acid glucosides
  - Sampling and analysis of condensed buttermilk
- Fertilizers
  - Sampling
  - Phosphoric acid
  - Moisture
  - Nitrogen
  - Magnesium and manganese
  - Acid- and base-forming quality
  - Potash and platinum recovery methods
  - Sulfur
  - Copper and zinc
  - Boron
- Insecticides and fungidicides
  - Fluorine compounds
  - Rodenticides
  - Nicotine and nornicotine
  - DDT
- Disinfectants
- Leathers and tanning materials
- Plants
  - Sampling
  - Iodine and boron
  - Carbohydrates
  - Zinc
  - Copper and cobalt
  - Carotene
- Soils and liming materials
  - Hydrogen-ion concentration of soils
  - Boron and fluorine
  - Zinc and copper
  - Exchangeable calcium and magnesium
  - Exchangeable hydrogen
  - Exchangeable potassium
  - Phosphorus
- Standard solutions
  - Potassium dichromate solutions
  - Thiocyanate solutions
  - Potassium permanganate solutions
  - Buffer solutions
  - Bromide-bromate solutions
  - Titanium trichloride solutions
- Vitamins
  - Vitamin A
  - Vitamin B_1
  - Vitaman C
  - Vitamin D—milk
  - Vitamin D—poultry
  - Riboflavin (fluorometric)
  - Nicotinic acid
  - Carotene
  - Pantothenic acid
  - Folic acid

SUBCOMMITTEE B: HARRY J. FISHER (1948), (Conn. Agricultural Experiment Station, New Haven, Conn.), *Chairman;* G. R. CLARK (1950), and F. H. WILEY (1952).

- Naval stores
- Radioactivity
  - Quantum counter
  - Analysis by radon measurement and alpha particle counting
- Spectrographic methods
- Vegetable drugs and their derivatives
  - Chemical methods for ergot alkaloids
  - Physostigmine in ointments
  - Theobromine and phenobarbital
  - Aminopyrine, ephedrine, and phenobarbital
  - Quinine
  - Ephedrine
  - Spirit of camphor
  - Chemical methods for penicillin
- Synthetic drugs
  - Methylene blue
  - Sulfanilamide derivatives
  - Atabrine (chinacrin, quinacrine)
  - Demerol
  - Propadrine hydrochloride
  - Carbromal
  - Dihydrocodeinone
  - Butacaine sulfate
  - Spectrophotometric methods
  - Trichloroethylene
  - Thiouracil
- Miscellaneous drugs
  - Microchemical tests for alkaloids and synthetics
  - Mercury compounds
  - Separation of bromides, chlorides, and iodides
  - Organic iodides
  - Compound ointment of benzoic acid
  - Alkali metals
  - Glycols and related compounds
  - Preservatives and bacteriostatic agents in ampul solutions
  - Phosphorus, calcium, and iron in vitamin preparations
  - Iodine
  - Superheated steam in separation of drugs
  - Estrone and estradiol
  - Chromatographic separation of drugs
- Drug bioassays
  - Enteric coatings
  - Ergometrine (ergonovine)
  - Digitalis preparations
- Cosmetics and coal-tar colors
  - Alkalies in cuticle removers
  - Cosmetic creams
  - Cosmetic powders
  - Cosmetic skin lotions
  - Deodorants and anti-perspirants
  - Depilatories
  - Hair dyes and rinses
  - Hair straighteners
  - Mascara, eyebrow pencils, and eye shadow
  - Mercury salts in cosmetics
  - Moisture in cosmetics
  - Pyrogallol in hair dyes
  - Acetates, carbonates, halides, and sulfates in certified coal-tar colors
  - Buffers and solvents in titanium trichloride titrations
  - Ether extract in coal-tar colors
  - Halogens in halogenated fluoresceins
  - Identification of certified coal-tar colors
  - Volatile amine intermediates in coal-tar colors
  - Non-volatile unsulfonated amine intermediates in coal-tar colors
  - Sulfonated amine intermediates
  - Unsulfonated phenolic intermediates
  - Sulfonated phenolic intermediates
  - Intermediates derived from phthalic acid
  - Mixtures of coal-tar colors for drug and cosmetic use
  - Lakes and pigments
  - Spectrophotometric testing of coal-tar colors
  - Subsidiary dyes in D&C colors

SUBCOMMITTEE C: C. S. LADD (1948) (Production and Marketing Administration, Washington 25, D. C.), *Chairman;* JOSEPH CALLAWAY (1950), and P. A. CLIFFORD (1952).

- Processed vegetable products
  - Quality factors
  - Moisture in dried vegetables
  - Catalase in frozen vegetables
- Fill of container methods (foods, drugs, and cosmetics)
- Coffee and tea
  - Chlorogenic acid in coffee
  - Caffeine in coffee extracts
- Coloring matters in foods
- Dairy products
  - Phosphatase test in dairy products
  - Ash in milk and evaporated milk
  - Sampling, fat, and moisture in cheese
  - Frozen desserts (gelatine and gums)
  - Frozen desserts (composition)
  - Chlorine in milk
  - Acidity of milk
  - Preparation of butter samples
  - Tests for reconstituted milk
  - Sour serum test
- Eggs and egg products
  - Added glycerol
  - Acidity of fat
- Extraneous materials in foods and drugs
  - Drugs, spices, and miscellaneous materials
  - Dairy products
  - Nut products and confectionery
  - Canned foods, cereal products, and eggs
  - Fruit products and beverage materials
  - Vegetable products
- Decomposition in foods
  - Fish products
  - Dairy products
  - Shellfish
  - Fruits
- Gelatine, dessert preparations, and mixes
  - Jelly strength
- Fish and other marine products
  - Total solids and ether extract
- Gums in foods
  - Cheese
  - Mayonnaise and French dressing
  - Frozen desserts
  - Starchy foods
  - Pectin-containing foods
  - Cacao products
- Meat and meat products
  - Dried skim milk and soybean flour in meat products
- Metals, other elements, and residues in foods
  - Cadmium
  - Copper
  - Zinc
  - Mercury
  - DDT as spray residue on foods
  - DDT in canned foods
- Microbiological methods
  - Canned fishery products
  - Canned meats
  - Canned tomatoes and other acid vegetable and fruit products
  - Eggs and egg products
  - Nuts and nut products
  - Frozen fruits and vegetables
- Microchemical methods
- Nuts and nut products
- Oils, fats, and waxes
  - Unsaponifiable matter
  - Peanut oil
  - Stability of fats
  - Antioxidants
- Preservatives and artificial sweeteners
  - Benzoate of soda and esters of benzoic acid
  - Saccharin
  - Quarternary ammonium compounds
  - Monochloracetic acid
  - Dichloroacetic acid
  - Formaldehyde
  - Mold-inhibitors, propionates
  - Diacatates
  - Thiourea
- Spices and other condiments
  - Vinegar
  - Volatile oil in spices
  - Starch, sugar, and ash in mustards
  - Starch in salad dressing

SUBCOMMITTEE D: C. S. FERGUSON (1948) (State Department Public Health, Boston, Mass.), *Chairman;* KENNETH L. MILSTEAD (1950), and J. WALTER SALE (1952).

- Alcoholic beverages
  - Malt
  - Diastatic activity and soluble starches
  - Hops
  - Yeast (total and yeast solids)
  - Brewing sugars, sirups, wort, spent grains, and yeast
  - Fermentable extracts in brewing sugars and sirups
  - Beer
  - Acidity and *p*H of beer
  - Inorganic elements in beer
  - Color and turbidity in beer
  - Carbon dioxide in beer
  - Distilled spirits
  - Spectrophotometric examination of wines and distilled spirits
  - Chromatographic absorption of wines
  - Wine
  - Methanol in wines and distilled liquors
  - Cordials and liqueurs
- Cacao products
  - Lecithin
  - Malt solids
  - Pectic acid
  - Cacao ingredients
  - Lactose
  - Fat
- Cereal foods
  - Starch in raw and cooked cereals
  - Fat acidity in grain, flour, corn meal, and whole wheat flour
  - Sugar in bread and other cereal foods
  - Benzoyl peroxide in flour
  - Carbon dioxide in self-rising flour
  - Milk solids and butterfat in bread
  - Proteolytic activity of flour
  - Soybean flour
  - Soybean flour in foods (immunological tests)
  - Phosphated flour
  - Noodles
  - Baked products (moisture, ash, protein, fat, and crude fiber)
  - Moisture in self-rising flour, and in pancake, waffle, and doughnut flours
  - Bromates in flour
  - Apparent viscosity measurement
  - Phosphorus
  - Iron in cornmeal and macaroni products
- Baking powders and baking chemicals
  - Carbon dioxide
- Flavors and non-alcoholic beverages
  - Beta-ionone
  - Lemon oils and extracts
  - Organic solvents in flavors
  - Glycerol, vanillin, and coumarin in vanilla and imitation vanilla
  - Emulsion flavors
  - Maple flavor concentrate and imitations
  - Diacetyl
- Fruits and fruit products
  - Titration of acids
  - Fruit acids
  - Fruit and sugar in frozen fruit
  - Water-insoluble solids
- Sugars and sugar products
  - Unfermented reducing substances in molasses
  - Drying methods
  - Densimetric and refractometric methods
  - Honey
  - Confectionery
  - Reducing sugars
  - Corn sirup and corn sugar
  - Color and turbidity in sugar products
- Waters, brine, and salt
  - Boron in water
  - Fluorine in salt

FAT IN COOKED ANIMAL FEEDS CONTAINING CEREALS:
S. B. Randle, Agricultural Experiment Station, Lexington 29, Ky.
CRUDE FAT OR ETHER EXTRACT:
J. J. Taylor, Department of Agriculture, Tallahassee, Fla.
ACTIVITY OF YEAST:
H. C. Schaefer, Ralston Purina Co., St. Louis, Mo.
MICROSCOPIC EXAMINATION:
H. J. Witteveen, State Department of Agriculture, Dairy, and Food, St. Paul, Minn.
FLUORINE:
D. M. Doty, Purdue University, Lafayette, Ind.
MINERAL CONSTITUENTS OF MIXED FEEDS:
J. L. St. John, Agricultural Experiment Station, Pullman, Wash.
CRUDE FIBER:
W. L. Hunter, Department of Agriculture, Sacramento 14, Calif.
PROTEIN EVALUATION IN FISH AND ANIMAL PRODUCTS:
Frank J. Kokoski, N. Y. Agricultural Experiment Station, Geneva, N. Y.
CRUDE PROTEIN (CATALYSIS):
R. C. Berry, State Department of Agriculture and Immigration, Richmond 19, Va.
HYDROCYANIC ACID GLUCOSIDES:
E. W. Constable, State Department of Agriculture, Raleigh, N. C.
SAMPLING AND ANALYSIS OF CONDENSED BUTTERMILK:
R. E. Bergman, State Department of Agriculture, St. Paul, Minn.

FERTILIZERS:
Referee: F. W. Quackenbush, Agricultural Experiment Station, Lafayette, Ind.
SAMPLING:
H. R. Allen, Agricultural Experiment Station, Lexington 29, Ky.
PHOSPHORIC ACID:
K. D. Jacob, Plant Industry Station, Beltsville, Md.
MOISTURE:
W. L. Hill, Plant Industry Station, Beltsville, Md.
NITROGEN:
M. P. Etheredge, State Department of Agriculture, State College, Miss.
MAGNESIUM AND MANGANESE:
John B. Smith, Agricultural Experiment Station, Kingston, R. I.
ACID- AND BASE-FORMING QUALITY:
E. W. Constable
POTASH AND PLATINUM RECOVERY METHODS:
O. W. Ford, Purdue University, Lafayette, Ind.
SULFUR:
Gordon Hart, Department of Agriculture, Tallahassee, Fla.
COPPER AND ZINC:
W. Y. Gary, Department of Agriculture, Tallahassee, Fla.
BORON:
Stacey Randle, Agricultural Experiment Station, New Brunswick, N. J.

INSECTICIDES AND FUNGICIDES:
Referee: J. J. T. Graham, Production and Marketing Administration, Insecticide Division, Beltsville, Md.
FLUORINE:
C. G. Donovan, Production and Marketing Administration, Insecticide Division, Beltsville, Md.

RODENTICIDES:
John W. Elmore, Department of Agriculture, Sacramento 14, Calif.
NICOTINE AND NORNICOTINE:
C. V. Bowen, Bur. Entomology and Plant Quarantine, Beltsville, Md.
DDT:
E. E. Fleck, Bur. Entomology and Plant Quarantine, Beltsville, Md.

DISINFECTANTS:
Referee: C. M. Brewer, Food and Drug Administration, Washington 25, D. C.

LEATHERS AND TANNING MATERIALS:
Referee: I. D. Clarke, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia 18, Pa.

PLANTS:
Referee: E. J. Miller, Agricultural Experiment Station, Lansing, Mich.
SAMPLING:
E. J. Miller
IODINE AND BORON:
J. S. McHargue, Agricultural Experiment Station, Lexington 29, Ky.
CARBOHYDRATES:
J. T. Sullivan, U. S. Regional Pasture Research Laboratory, State College, Pa.
ZINC:
E. J. Benne, Agricultural Experiment Station, East Lansing, Mich.
COPPER AND COBALT:
Lillian I. Butler, Bur. Entomology and Plant Quarantine, Beltsville, Md.
CAROTENE:
E. J. Benne

SOILS AND LIMING MATERIALS:
Referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville 16 Tenn.
HYDROGEN-ION CONCENTRATION OF SOILS:
W. T. McGeorge, Agricultural Experiment Station, Tucson, Ariz.
BORON AND FLUORINE
J. S. McHargue, Agricultural Experiment Station, Lexington 29, Ky.
ZINC AND COPPER:
L. H. Rogers, Agricultural Experiment Station, Gainesville, Fla.
EXCHANGEABLE CALCIUM AND MAGNESIUM:
W. M. Shaw, Agricultural Experiment Station, Knoxville 16, Tenn.
EXCHANGEABLE HYDROGEN:
W. M. Shaw
EXCHANGEABLE POTASSIUM:
Ivan E. Miles, N. C. Department of Agriculture, Raleigh, N. C.
PHOSPHORUS:
L. A. Dean, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Md.

STANDARD SOLUTIONS:
Referee: H. G. Underwood, Food and Drug Administration, Chicago 7, Ill.
POTASSIUM DICHROMATE SOLUTIONS:
Lester LaGrange, Food and Drug Administration, New Orleans, La.
THIOCYANATE SOLUTIONS:
E. C. Deal, Food and Drug Administration, New Orleans 16, La.
POTASSIUM PERMANGANATE SOLUTIONS:
R. E. Duggan, Food and Drug Administration, New Orleans 16, La.

BUFFER SOLUTIONS:
George G. Manov, National Bureau of Standards, Washington 25, D. C.
BROMIDE-BROMATE SOLUTIONS:
Halver C. Van Dame, Food and Drug Administration, Cincinnati 2, Ohio
TITANIUM TRICHLORIDE SOLUTIONS:
Juanita E. Breit, Food and Drug Administration, Cincinnati 2, Ohio

VITAMINS:
Referee: Chester D. Tolle, Food and Drug Administration, Washington 25, D. C.
VITAMIN A:
J. B. Wilkie, Food and Drug Administration, Washington 25, D. C.
VITAMIN B_1:
O. L. Kline, Food and Drug Administration, Washington 25, D. C.
VITAMIN C:
W. L. Hall, Food and Drug Administration, Washington 25, D. C.
VITAMIN D—POULTRY:
Chester D. Tolle
RIBOFLAVIN (FLUOROMETRIC):
H. W. Loy, Jr., Food and Drug Administration, Washington 25, D. C.
NICOTINIC ACID:
F. M. Strong, University of Wisconsin, Madison, Wis.
CAROTENE:
F. W. Quackenbush
PANTOTHENIC ACID:
H. W. Loy, Jr.
FOLIC ACID:
Laura Flynn, College of Agriculture, University of Missouri, Columbia, Mo.

Subcommittee B

NAVAL STORES:
Referee: V. E. Grotlisch, Production and Marketing Administration, Naval Stores Division, Washington 25, D. C.

RADIOACTIVITY:
Referee L. F. Curtiss, National Bureau of Standards, Washington 25, D. C.
QUANTUM COUNTER:
Anna E. Mix, Food and Drug Administration, Washington 25, D. C.
ANALYSIS BY RADON MEASUREMENT AND ALPHA PARTICLE COUNTING:
Francis J. Davis, National Bureau of Standards, Washington, 25, D. C.

SPECTROGRAPHIC METHODS:
Referee: W. T. Mathis, Connecticut Agricultural Experiment Station, New Haven, Conn.

VEGETABLE DRUGS AND THEIR DERIVATIVES:
Referee: P. S. Jorgensen, Food and Drug Administration, Seattle 4, Wash.
CHEMICAL METHODS FOR ERGOT ALKALOIDS:
Joseph Levine, U. S. Bureau of Narcotics, Washington 25, D. C.
PHYSOSTIGMINE IN OINTMENTS:
M. L. Dow, Food and Drug Administration, St. Louis 1, Mo.
THEOBROMINE AND PHENOBARBITAL:
Daniel Banes, Food and Drug Administration, Chicago 7, Ill.
AMINOPYRINE, EPHEDRINE, AND PHENOBARBITAL:
C. D. Wright, Food and Drug Administration, Washington 25, D. C.

QUININE:
D. J. Miller, Food and Drug Administration, Buffalo 3, N. Y.
EPHEDRINE:
L. H. Welsh, Food and Drug Administration, Washington 25, D. C.
SPIRIT OF CAMPHOR:
H. W. Conroy, Food and Drug Administration, Kansas City 6, Mo.
CHEMICAL METHODS FOR PENICILLIN:
H. Fischbach, Food and Drug Administration, Washington 25, D. C.

SYNTHETIC DRUGS:
Referee: F. C. Sinton, Food and Drug Administration, New York 14, N. Y.
METHYLENE BLUE:
H. O. Moraw, Food and Drug Administration, Chicago 7, Ill.
SULFANILAMIDE DERIVATIVES:
A. E. Sidwell, Jr., American Medical Association, Chicago, Ill.
ATABRINE (CHINACRIN, QUINACRINE):
H. C. Heim, Food and Drug Administration, San Francisco, Calif.
DEMEROL:
Merlin Mundell, P.O. Box 793, Kilgore, Tex.
PROPADRINE HYDROCHLORIDE:
R. D. Stanley, Food and Drug Administration, Chicago 7, Ill.
CARBROMAL:
R. Hyatt, Food and Drug Administration, Cincinnati 2, Ohio
DIHYDROCODEINONE:
F. J. McNall, Food and Drug Administration, Cincinnati 2, Ohio
BUTACAINE SULFATE:
L. H. Welsh, Food and Drug Administration, Washington 25, D. C.
SPECTROPHOTOMETRIC METHODS:
J. Carol, Food and Drug Administration, Washington 25, D. C.
TRICHLORETHYLENE:
Gordon Smith, Food and Drug Administration, New York 14, N. Y.
THIOURACIL:
Muriel Drucker, Food and Drug Administration, New York 14, N. Y.

MISCELLANEOUS DRUGS:
Referee: Iman Schurman, Food and Drug Administration, Chicago 7, Ill.
MICROCHEMICAL TESTS FOR ALKALOIDS AND SYNTHETICS:
W. V. Eisenberg, Food and Drug Administration, Washington 25, D. C
MERCURY COMPOUNDS:
M. W. Green, American Pharmaceutical Assn., Washington 25, D. C.
SEPARATION OF BROMIDES, CHLORIDES, AND IODIDES:
N. E. Freeman, Food and Drug Administration, Atlanta 3, Ga.
ORGANIC IODIDES:
F. A. Rotondaro, Bristol Laboratories, Syracuse, N. Y.
COMPOUND OINTMENT OF BENZOIC ACID:
W. F. Kunke, Food and Drug Administration, Chicago 7, Ill.
ALKALI METALS:
H. F. O'Keefe, Food and Drug Administration, Chicago 7, Ill.
GLYCOLS AND RELATED COMPOUNDS:
Harry Isacoff, Food and Drug Administration, New York 14, N. Y.
PRESERVATIVES AND BACTERIOSTATIC AGENTS IN AMPUL SOLUTIONS:
C. N. Jones, Food and Drug Administration, New York 14, N. Y.
PHOSPHORUS, CALCIUM, AND IRON IN VITAMIN PREPARATIONS:
Daniel Banes, Food and Drug Administration, Chicago 7, Ill.

IODINE:
Sam Fine, Food and Drug Administration, Cincinnati 2, Ohio
SUPERHEATED STEAM IN SEPARATION OF DRUGS:
Urban Oakdale, Food and Drug Administration, Chicago 7, Ill.
ESTRONE AND ESTRADIOL:
J. C. Molliter, Food and Drug Administration, Washington 25, D. C.
CHROMATOGRAPHIC SEPARATION OF DRUGS:
T. E. Eble, Food and Drug Administration, Washington 25, D. C.

DRUG BIOASSAYS:
Referee: B. J. Vos, Jr., Food and Drug Administration, Washington 25, D. C.
ENTERIC COATINGS:
H. A. Braun, Food and Drug Administration, Washington 25, D. C.
ERGOMETRINE (ERGONOVINE):
B. J. Vos., Jr.
DIGITALIS PREPARATIONS:
B. J. Vos, Jr.

COSMETICS AND COAL-TAR COLORS:
Referee: G. R. Clark, Food and Drug Administration, Washington 25, D. C.
COSMETIC CREAMS:
C. F. Bruening, Food and Drug Administration, Baltimore 2, Md.
COSMETIC POWDERS:
George McClellan, Food and Drug Administration, New Orleans 16, La.
COSMETIC SKIN LOTIONS:
H. R. Bond, Food and Drug Administration, Kansas City 6, Mo.
DEODORANTS AND ANTI-PERSPIRANTS:
S. H. Newburger, Food and Drug Administration, Washington 25, D. C.
DEPILATORIES:
S. H. Newburger
HAIR DYES AND RINSES:
S. H. Newburger
MASCARA, EYEBROW PENCILS, AND EYE SHADOW:
Paul W. Jewel, Max Factor and Company, Hollywood, Calif.
MERCURY SALTS IN COSMETICS:
Gertrude J. Lowell, Food and Drug Administration, New York 14, N. Y.
MOISTURE IN COSMETICS:
J. F. Weeks, Food and Drug Administration, New Orleans 16, La.
PYROGALLOL IN HAIR DYES:
C. R. Joiner, Food and Drug Administration, St. Louis 1, Mo.
ACETATES, CARBONATES, HALIDES, AND SULFATES IN CERTIFIED COAL-TAR COLORS:
A. T. Schram, National Aniline Division, P.O. Box 975, Buffalo 5, N. Y.
BUFFERS AND SOLVENTS IN TITANIUM TRICHLORIDE TITRATIONS:
S. S. Forrest, Food and Drug Administration, Washington 25, D. C.
ETHER EXTRACT IN COAL-TAR COLORS:
S. S. Forrest
HALOGENS IN HALOGENATED FLUORESCEINS:
K. A. Freeman, Food and Drug Administration, Washington 25, D. C.
IDENTIFICATION OF CERTIFIED COAL-TAR DYES:
K. A. Freeman
VOLATILE AMINE INTERMEDIATES IN COAL-TAR COLORS:
Alice B. Caemmerer, Food and Drug Administration, Washington 25, D. C.

NON-VOLATILE UNSULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS:
K. A. Freeman

SULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS:
K. A. Freeman

UNSULFONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS:
H. Holtzman, Ansbacher-Siegle Corporation, Rose Bank, Staten Island, N. Y.

SULFONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS:
K. A. Freeman

INTERMEDIATES DERIVED FROM PHTHALIC ACID:
K. A. Freeman

MIXTURES OF COAL-TAR COLORS FOR DRUGS AND COSMETIC USE:
W. C. Bainbridge, H. Kohnstamm Company, Brooklyn 31, N. Y.

LAKES AND PIGMENTS:
K. A. Freeman

SPECTROPHOTOMETRIC TESTING OF COAL-TAR COLORS:
Rachel N. Sclar, Food and Drug Administration, Washington 25, D. C.

SUBSIDIARY DYES IN D&C COLORS:
L. Koch, H. Kohnstamm and Company, Brooklyn 31, N. Y.

Subcommittee C

PROCESSED VEGETABLE PRODUCTS:
Referee: V. B. Bonney, Food and Drug Administration, Washington 25, D. C.

QUALITY FACTORS:
Samuel C. Oglesby, Food and Drug Administration, Washington 25, D. C.

MOISTURE IN DRIED VEGETABLES:
B. Makover, Western Regional Research Laboratory, U. S. Department of Agriculture, Albany 6, Calif.

CATALASE IN FROZEN VEGETABLES:
Hans Lineweaver, Western Regional Research Laboratory, U. S. Department of Agriculture, Albany 6, Calif.

FILL OF CONTAINER METHODS (FOODS, DRUGS, AND COSMETICS):
Referee: Sumner C. Rowe, Food and Drug Administration, Washington 25, D. C.

COFFEE AND TEA:
Referee: Harry J. Fisher, Agricultural Experiment Station, New Haven 4, Conn.

COLORING MATTERS IN FOODS:
Referee: C. F. Jablonski, Food and Drug Administration, New York 14, N. Y.

DAIRY PRODUCTS:
Referee: Guy G. Frary, State Chemical Laboratory, Vermillion, S. Dak.

PHOSPHATASE TEST IN DAIRY PRODUCTS:
George P. Sanders, Bur. Dairy Industry, U. S. Department of Agriculture, Washington 25, D. C.

ASH IN MILK AND EVAPORATED MILK:
Guy G. Frary

SAMPLING, FAT, AND MOISTURE IN CHEESE:
W. Horwitz, Food and Drug Administration, Minneapolis 1, Minn.

FROZEN DESSERTS (GELATINE AND GUMS):
F. Leslie Hart, Food and Drug Administration, Los Angeles, Calif.

CHLORINE IN MILK:
W. H. King, State Department of Health, New Orleans 7, La.
ACIDITY OF MILK:
Guy G. Frary
PREPARATION OF BUTTER SAMPLES:
H. J. Meuron, Food and Drug Administration, San Francisco 2, Calif.
TESTS FOR RECONSTITUTED MILK:
W. H. King
SOUR SERUM TEST:
Henry J. Hoffman, Minnesota Dept. of Agriculture, St. Paul, Minn.

EGGS AND EGG PRODUCTS:
Referee: F. J. McNall, Food and Drug Administration, Cincinnati 2, Ohio
ADDED GLYCEROL:
George E. Keppel, Food and Drug Administration, Minneapolis 1, Minn.
ACIDITY OF FAT:
F. J. McNall

EXTRANEOUS MATERIALS IN FOODS AND DRUGS:
Referee: J. D. Wildman, Food and Drug Administration, Washington 25, D. C.
DRUGS, SPICES, AND MISCELLANEOUS MATERIALS:
W. V. Eisenberg, Food and Drug Administration, Washington 25, D. C.
DAIRY PRODUCTS:
J. D. Wildman
NUT PRODUCTS AND CONFECTIONERY:
W. G. Helsel, Food and Drug Administration, Washington 25, D. C.
CANNED FOODS, CEREAL PRODUCTS, AND EGGS:
K. L. Harris, Food and Drug Administration, Washington 25, D. C.
FRUIT PRODUCTS AND BEVERAGE MATERIALS:
F. A. Hodges, Food and Drug Administration, Washington 25, D. C.
VEGETABLE PRODUCTS:
F. R. Smith, Food and Drug Administration, Washington 25, D. C.

DECOMPOSITION IN FOODS:
Referee: W. I. Patterson, Food and Drug Administration, Washington 25, D. C.
FISH PRODUCTS:
Fred Hillig, Food and Drug Administration, Washington 25, D. C.
DAIRY PRODUCTS:
Fred Hillig
SHELLFISH:
R. E. Duggan, Food and Drug Administration, New Orleans 16, La.
FRUITS:
T. H. Harris, Food and Drug Administration, Washington 25, D. C.

GELATINE, DESSERT PREPARATIONS, AND MIXES:
Referee: Sumner C. Rowe, Food and Drug Administration, Washington 25, D. C.
JELLY STRENGTH:
Paul A. Kind, Kind-Knox Gelatine Co., Camden, N. J.

FISH AND OTHER MARINE PRODUCTS:
Referee: A. M. Allison, Food and Drug Administration, Boston 10, Mass.
TOTAL SOLIDS AND ETHER EXTRACT:
Menno D. Voth, Food and Drug Administration, Boston 10, Mass.

GUMS IN FOODS:
Referee: F. Leslie Hart, Food and Drug Administration, Los Angeles 15, Calif.
CHEESE:
M. J. Gnagy, Food and Drug Administration, Los Angeles 15, Calif.
FROZEN DESSERTS
F. Leslie Hart
STARCHY FOODS:
Sutton Redfern, Fleischmann Laboratories, New York 51, N. Y.
CACAO PRODUCTS:
Flora Y. Mendelsohn, Food and Drug Administration, Los Angeles 15, Calif.

MEAT AND MEAT PRODUCTS:
Referee: Roger M. Mehurin, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.

DRIED SKIM MILK AND SOYBEAN FLOUR IN MEAT PRODUCTS:
O. L. Bennett, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.

METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS:
Referee: H. J. Wichmann, Food and Drug Administration, Washington 25, D.C.
CADMIUM:
A. K. Klein, Food and Drug Administration, Washington 25, D. C.
COPPER:
Gordon H. Bendix, Continental Can Company, Inc., Chicago, Ill.
ZINC:
O. R. Alexander, American Can Company, Maywood, Ill.
DDT AS SPRAY RESIDUE ON FOODS:
R. H. Carter, Bureau of Entomology and Plant Quarantine, Beltsville, Md.
DDT IN CANNED FOODS:
C. J. Tressler, National Canners' Association, Washington, D. C.

MICROBIOLOGICAL METHODS:
Referee: G. G. Slocum, Food and Drug Administration, Washington 25, D. C.
CANNED MEATS:
M. L. Laing, Armour & Company, Chicago 9, Ill.
CANNED TOMATOES AND OTHER ACID VEGETABLE AND FRUIT PRODUCTS:
B. A. Linden, Food and Drug Administration, Washington 25, D. C.
EGGS AND EGG PRODUCTS:
M. T. Bartram, Food and Drug Administration, Washington 25, D. C.
NUTS AND NUT PRODUCTS:
William R. North, Food and Drug Administration, Washington 25, D. C.
FROZEN FRUITS AND VEGETABLES:
H. E. Goresline, Production and Marketing Administration, Poultry Division, Washington 25, D. C.

MICROCHEMICAL METHODS:
Referee: C. O. Willits, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia, Pa.
ELEMENTAL ANALYSIS:
C. L. Ogg, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia, Pa.

NUTS AND NUT PRODUCTS:
Referee: A. M. Henry, Food and Drug Administration, Atlanta 3, Ga.

OILS, FATS, AND WAXES:
Referee: J. Fitelson, Food and Drug Administration, New York 14, N. Y.
UNSAPONIFIABLE MATTER:
Gardner Kirsten, Food and Drug Administration, New York 14, N. Y.
PEANUT OIL:
Gardner Kirsten
STABILITY OF FATS:
E. F. Steagall, Food and Drug Administration, Washington 25, D. C.
ANTIOXIDANTS:
William L. Potter, Eastern Regional Laboratory, U. S. Department of Agriculture, Philadelphia 18, Pa.

PRESERVATIVES AND ARTIFICIAL SWEETENERS:
Referee: Margarethe Oakley, State Department of Health, Baltimore 18, Md.
BENZOATE OF SODA AND ESTERS OF BENZOIC ACID:
W. J. McCarthy, U. S. Food and Drug Administration, Cincinnati 2, Ohio
SACCHARIN:
Margarethe Oakley
QUARTERNARY AMMONIUM COMPOUNDS:
John B. Wilson, Food and Drug Administration, Washington 25, D. C.
MONOCHLORACETIC ACID:
John B. Wilson
DICHLOROACETIC ACID:
A. Bruening, Food and Drug Administration, Baltimore 2, Md.
MOLD-INHIBITORS, PROPIONATES:
L. H. McRoberts, Food and Drug Administration, San Francisco 2, Calif.
THIOUREA:
H. I. Macomber, Food and Drug Administration, Baltimore 2, Md.

SPICES AND OTHER CONDIMENTS:
Referee: S. Alfend, Food and Drug Administration, St. Louis 1, Mo.
VINEGAR:
J. H. C. Loughrey, Food and Drug Administration, Boston, Mass.
VOLATILE OIL IN SPICES:
Dan Unger, Food and Drug Administration, New York 14, N. Y.
STARCH, SUGAR, AND ASH IN MUSTARDS:
F. M. Garfield, Food and Drug Administration, St. Louis 1, Mo.
STARCH IN SALAD DRESSING:
Sam D. Fine, Food and Drug Administration, Cincinnati 2, Ohio

Subcommittee D

ALCOHOLIC BEVERAGES:
Referee: J. Walter Sale, Food and Drug Administration, Washington 25, D. C.
MALT:
Christian Rask, Albert Schwill Company, Chicago, Ill.
DIASTATIC ACTIVITY AND SOLUBLE STARCHES:
Allan D. Dickson, University of Wisconsin, Madison, Wis.
HOPS:
D. E. Bullis, Oregon State College, Corvallis, Ore.
YEAST (TOTAL AND YEAST SOLIDS):
Robert I. Tenney, Wahl-Henius Inst., 64 E. Lake St., Chicago, Ill.

BREWING SUGARS, SIRUPS, WORT, SPENT GRAINS, AND YEAST:
Stephen Laufer, Schwarz Laboratories, New York 17, N. Y.
FERMENTABLE EXTRACTS IN BREWING SUGARS AND SIRUPS:
P. P. Gray, Wallerstein Laboratories, New York 16, N. Y.
BEER:
H. W. Rohde, Schlitz Brewing Company, Milwaukee 1, Wis.
ACIDITY AND *p*H OF BEER:
Kurt Becker, Siebel Institute, Chicago, Ill.
INORGANIC ELEMENTS IN BEER:
Gordon H. Bendix, Continental Can Company, Inc., Chicago, Ill.
COLOR AND TURBIDITY IN BEER:
B. H. Nissen, Anheuser-Busch, Inc., St. Louis, Mo.
CARBON DIOXIDE IN BEER:
Irwin Stone, Wallerstein Laboratories, New York 16, N. Y.
DISTILLED SPIRITS:
G. F. Beyer, Bureau of Internal Revenue, Washington 25, D. C.
SPECTROPHOTOMETRIC EXAMINATION OF WINES AND DISTILLED SPIRITS:
G. F. Beyer
CHROMATOGRAPHIC ABSORPTION OF WINES:
Peter Valaer
WINE
Peter Valaer
METHANOL IN WINES AND DISTILLED LIQUORS:
Louis Arrigoni, University of Washington, Seattle, Wash.
CORDIALS AND LIQUEURS:
John B. Wilson, Food and Drug Administration, Washington 25, D. C.

CACAO PRODUCTS:
Referee: W. O. Winkler, Food and Drug Administration, Washington 25, D. C.
LECITHIN:
J. H. Bornmann, Food and Drug Administration, Chicago 7, Ill.
MALT SOLIDS:
E. W. Meyers, Hershey Chocolate Company, Hershey, Pa.
PECTIC ACID:
W. O. Winkler
CACAO INGREDIENTS:
W. O. Winkler
LACTOSE:
Frank V. Kenney, Jr., Walter Baker Co., Dorchester 24, Mass.
FAT:
Carl Stone, Food and Drug Administration, Cincinnati, Ohio

CEREAL FOODS
Referee: V. E. Munsey, Food and Drug Administration, Washington 25, D. C.
FAT ACIDITY IN GRAIN, FLOUR, CORN MEAL, AND WHOLE WHEAT FLOUR:
Lawrence Zeleny, Agricultural Research Center, Beltsville, Md.
SUGAR IN BREAD AND OTHER CEREAL FOODS:
N. H. Walker, National Biscuit Company, New York 11, N. Y.
BENZOYL PEROXIDE IN FLOUR:
V. E. Munsey
CARBON DIOXIDE IN SELF-RISING FLOUR:
R. A. Barackman, Victor Chemical Works, Chicago Heights, Ill.
MILK SOLIDS AND BUTTERFAT IN BREAD:
V. E. Munsey

PROTEOLYTIC ACTIVITY OF FLOUR:
Sutton Redfern, Fleischmann Laboratories, New York 51, N. Y.
SOYBEAN FLOUR:
W. L. Taylor, General Mills, Inc., Minneapolis, Minn.
PHOSPHATED FLOUR:
Frank H. Collins, Food and Drug Administration, Cincinnati 2, Ohio
NOODLES:
Leslie W. Ferris, Food and Drug Administration, Buffalo, N.Y.
BAKED PRODUCTS (MOISTURE, ASH, PROTEIN, FAT, AND CRUDE FIBER):
N. H. Walker
MOISTURE IN SELF-RISING FLOUR AND IN PANCAKE, WAFFLE, AND DOUGHNUT FLOURS:
E. F. Steagall, Food and Drug Administration, Washington 25, D. C.
BROMATES IN FLOUR:
W. F. Geddes, University of Minnesota, University Farm, St. Paul, Minn.
APPARENT VISCOSITY MEASUREMENT:
E. G. Bayfield, Standard Milling Company, 309 West Jackson Blvd., Chicago 6, Ill.
PHOSPHORUS:
V. E. Munsey
IRON IN CORNMEAL AND MACARONI PRODUCTS:
V. E. Munsey

BAKING POWDERS AND BAKING CHEMICALS:
Referee: V. E. Munsey, Food and Drug Administration, Washington 25, D. C.
CARBON DIOXIDE:
John E. Tatar, Standard Brands, Inc., 1015 Independence Blvd., Chicago, Ill.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:
Referee: John B. Wilson, Food and Drug Administration, Washington 25, D. C.
BETA-IONONE:
John B. Wilson
LEMON OILS AND EXTRACTS:
John B. Wilson
ORGANIC SOLVENTS IN FLAVORS:
R. D. Stanley, Food and Drug Administration, Chicago 7, Ill.
EMULSION FLAVORS:
John B. Wilson
DIACETYL:
John B. Wilson

FRUITS AND FRUIT PRODUCTS:
Referee: R. A. Osborn, Food and Drug Administration, Washington 25, D. C.
TITRATION OF ACIDS:
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PRESIDENT'S ADDRESS*

THE INFLUENCE OF FERTILIZERS IN PROMOTING CROP GROWTH

By WILLIAM H. ROSS (Division of Soils, Fertilizers, and Irrigation, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland)

The use of commercial fertilizers as a means for increasing the growth of crops was undertaken in England about 105 years ago. The production of fertilizers in the United States followed a decade or two later. Owing to the variable composition of the fertilizers produced in the early history of the industry, a demand soon developed for some knowledge of the quality of fertilizers offered for sale. In response to this demand, the State of Massachusetts took the lead in passing a fertilizer control law in 1873. The following year New Jersey enacted a similar law. Alabama, North Carolina, and other States followed with fertilizer control laws a few years later. The situation that prevailed after the passage of these laws was a peculiar one. The few chemists who were engaged at this time in the analysis and evaluation of fertilizers acted in complete independence of each other. Different methods of sampling and of analysis were used in the different fertilizer and control laboratories and there soon developed, as might be expected, a great deal of confusion and disagreement. This finally became so acute that in 1880 the Georgia Commissioner of Agriculture called a meeting of the officials of the largest fertilizer-consuming States for the purpose of securing more uniform and satisfactory methods for the analysis of commercial fertilizers. This meeting was held in Washington on July 28, and was attended by 20 delegates. Other meetings were subsequently held at Boston, Cincinnati, Atlanta, and Philadelphia. At the Philadelphia meeting in 1884, a motion was adopted that the work be continued as a permanent organization to be known as the Association of Official Agricultural Chemists. Officers were accordingly appointed and the meeting resolved into the first annual meeting of the Association. The first recommendations adopted by the new association related to methods for the determination of phosphoric acid, nitrogen, and potash in fertilizers.

The third meeting of the Association was the first to be held in Washington. Up to this time the entire proceedings of the Association had been given over to a discussion of methods for the analysis of fertilizers. In his Presidential address at this third meeting of the Association, Dr. Harvey W. Wiley recommended that the investigations of the Association be ex-

* Presented before the 60th Meeting of the Association of Official Agricultural Chemists, held at The Shoreham Hotel, Washington, D. C., October 14–16, 1946.

tended over a wide range of subjects and he expressed the opinion that every problem connected with chemical agricultural analysis was a proper subject for discussion at meetings. This recommendation was accepted and the following year reports were presented on the subject of feeding stuffs and dairy products in addition to fertilizers. Other subjects have been added to from time to time, until now the work of the Association comprises a total of 43 different subjects. As a result of these additions to the work of the Association the subject of fertilizers, which for several years comprised the entire proceedings of the Association, now occupies only a little more than two per cent of the space in the latest edition of the *Book of Methods*. This remarkable change in the relative attention given to fertilizers at the meetings of the Association does not mean that they are considered to be of less importance than before. It means rather that a much greater proportion of the total work of the Association is now given over to the development of methods for the analysis of materials other than fertilizers.

CONSUMPTION OF FERTILIZERS

The events of the past five years have demonstrated more than ever before the importance of fertilizers in the economic welfare of the nation. In 1884 when this Association was organized the consumption of fertilizers in the United States amounted to about 1,500,000 tons with an average plant food content of about 13 per cent. During the six years since the World War started, the consumption of fertilizers in this country increased from about 8,000,000 tons in 1939 to 13,500,000 tons in 1945, and that of liming materials from 9,000,000 to 24,000,000 tons. The average plant food content of fertilizers amounted to 18.5 per cent in 1939, and to 21.5 in 1945. These figures show that the increase in fertilizer consumption during the war years, when expressed in terms of plant food, was approximately equal to the maximum increase that took place during the preceding 50 years.

FERTILIZERS AND THE FOOD SUPPLY

The primary purpose in applying fertilizers to the soil is to bring about an increase in the growth of crops. During the war years many factors have operated to cause a world-wide shortage in the food supply and to greatly increase the demand for fertilizers. The World War interfered with the normal production of crops in great areas by interrupting normal transportation; by turning hundreds of thousands of farmers into soldiers; and by destroying draft power, farm machinery, and fertilizer plants. Seeds intended for planting were eaten because of the extreme shortage of food. A whole series of other disasters still further aggravated the effects of the war. Early in 1944 a serious drought spread over almost all of Latin America. This lack of rain extended to Australia and in the following year

to North Africa and parts of Europe. The United States, Canada, and limited areas in other parts of the world alone were spared. Upon these countries there fell the responsibility of doing all in their power to prevent a condition of almost world-wide famine.

During the first World War an attempt was made to increase the food supply by increasing the acreage of land under cultivation. This scheme proved disastrous in the Great Plains area and resulted in the formation of the "dust bowl." The possibility of another dust bowl was too real to be readily forgotten. This method of increasing the food supply, during World War II, was moreover an impractical one owing to the shortage of labor on farms. A plan had therefore to be devised that would bring about the maximum growth of crops with the minimum requirement of man power. The increased use of fertilizers was one means of accomplishing this result. Accordingly, the Department of Agriculture, the War Production Board, and other governmental agencies, as well as private industry, cooperated closely in efforts to insure supplies of fertilizers to meet the expanded requirements. While this cooperation of government and industry was successful in bringing about a marked increase in the consumption of fertilizers, as already explained, the acreage under cultivation remained fairly constant. Between 1939 and 1944 there was a small increase in acreage, but during the past two years the acreage under crops actually decreased slightly. The mean for these two years is approximately the same as that for the past 25 years.

FERTILIZERS AND SOIL FERTILITY

It is generally recognized that fertilizers will increase the growth of crops when properly used. This action has sometimes been explained on the theory that they force the soil without producing any lasting beneficial effect. Those who hold to this view believe that the more fertilizers are used the more the soil is forced and the more quickly it becomes exhausted. That the soils of the United States are becoming depleted or declining in fertility is a favorite view of certain agricultural scientists, and of many laymen, even of the present day. There is no doubt that the soil in certain local areas has decreased in fertility as a result of erosion, poor management, or for other reasons, but I do not know any way of proving whether the fertility of the soils of the United States as a whole is increasing or decreasing. Although a great deal has been written of late about the declining fertility of our soils, the idea is actually a very, very old one. To show that this is true I quote the following paragraph from a book written nearly 2,000 years ago by the distinguished Roman agriculturist, Columella:

"Again and again I hear leading men of our state condemning the unfruitfulness of the soil and the inclemency of the climate . . . as being harmful to crops; and some I hear reconciling the aforesaid complaints on the

ground that the soil is worn-out and exhausted by the overproduction of earlier days and can no longer furnish sustenance to mortals with its old-time benevolence. Such views I am convinced are far from the truth for it is a sin to suppose that Nature . . . is affected with barrenness as though with some disease, and it is unbecoming to a man of good judgement to believe that Earth . . . who is called the common mother of us all . . . has grown old in mortal fashion. Furthermore, I do not believe that such misfortunes come upon us as a result of the fury of the elements but rather because of our own fault."

TABLE 1.—*Average annual acre yield of corn, oats, and wheat in the United States by decades*

DECADE	CORN	OATS	WHEAT
	bushels	*bushels*	*bushels*
1866–1875	25.6	26.6	12.3
1876–1885	26.2	27.7	13.0
1886–1895	25.4	26.8	13.7
1896–1905	27.1	29.8	13.8
1906–1915	27.0	29.2	14.9
1916–1925	26.5	30.7	13.5
1926–1935	23.9	27.7	13.8
1936–1940[1]	26.0	30.0	13.8
1941–1945[1]	33.0	32.7	17.7
1946	37.1	34.6	17.2

[1] Five years.

In defense of Columella's views, it may be stated that the area about which he wrote is, at least, more productive now than it was 2,000 years ago.

FERTILIZERS AND SOIL PRODUCTIVITY

Although little can be said with authority on the permanence of the fertility of the average soil in the United States, the status of the productivity of the soil is a matter about which there is no question whatever. The productivity of the soil as determined by crop yields depends not only on the inherent fertility of the soil but also on such other factors as climate, farm management, quality of seed, and control of insects and diseases. Data on crop yields in this country have been collected with a marked degree of exactness over a period of 80 years. The principal basis of the crop estimates is the information collected every fifth year by the Bureau of the Census. Comparable data for the intervening years is collected by the Crop Reporting Service of the Bureau of Agricultural Economics. This service is operated by the U. S. Department of Agriculture in cooperation with all the 48 States, and with the help of about 300,000 voluntary crop reporters in all parts of the country.

In Tables 1 and 2 the average crop yields are given by decades for six of the leading crops in the United States. Table 1 shows that the acre yields of corn, oats, and wheat exhibited little or no upward trend during the first 7 decades. That the fluctuations noted were not due entirely to the rainfall is indicated by the fact that the precipitation for the corn belt and for the country as a whole had been both above and below the average during each 10-year period. During the war years the average-per-acre yields for all three crops showed a noticeable increase over any preceding period. As already explained, crop yields depend upon climate, farm

TABLE 2.—*Average annual acre yield of cotton, tobacco, and potatoes in the United States by decades*

DECADES	COTTON U.S.	TOBACCO U.S.	POTATOES	
			U.S.	MAINE
	pounds	*pounds*	*bushels*	*bushels*
1866–1875	164.6	746	87	107
1876–1885	172.0	729	83	108
1886–1895	182.3	729	81	131
1896–1905	190.6	795	88	143
1906–1915	192.2	826	100	210
1916–1925	161.4	785	102	219
1926–1935	179.1	790	111	262
1936–1940[1]	219.1	909	122	261
1941–1945[1]	260.4	1033	138	287
1946	230.7	1153	184	355

[1] Five years.

management, use of fertilizers, quality of seed, and control of insects and diseases. During the past 10 years the rainfall in the United States as a whole has been 2 per cent above normal. This small increase in rainfall would tend to increase production, but the extent to which this increase in precipitation was beneficial to crops can not be definitely stated. There was a decrease in labor on farms but this was offset, in part at least, by the longer working hours per day; by the greater use of disease-resistant varieties of seed; and by a definite increase in the use of insecticides and fungicides. Owing to the shortage of labor on farms it is unlikely that farm management was carried out more efficiently during this period than in previous years. Much progress has been made, however, in the development of improved varieties of corn, oats, and wheat. This is particularly true in the case of hybrid corn which has been used almost exclusively during the war years in the principal corn producing States. Of the acreage given over to these three crops more than half of the corn, about half of the oats, and considerably less than half of the wheat is fertilized. If fertilizers were in any way responsible for the increased yields that occurred during the past 5 or 6 years, it would be expected that the

TABLE 3.—*Average annual yield per acre of four of the principal crops of the United States in three groups of states by decades*

DECADE	CORN			WHEAT			OATS			POTATOES		
	VA., N.C., S.C., GA.	N.Y., N.J., OHIO, IND.	MINN., IOWA, NEBR., KAN.	VA., N.C., S.C., GA.	N.Y., N.J., OHIO, IND.	MINN., IOWA, NEBR., KAN.	VA., N.C., S.C., GA.	N.Y., N.J., OHIO, IND.	MINN., IOWA, NEB., KANS.	VA., N.C., S.C., GA.	N.Y., N.J., OHIO, IND.	MINN., IOWA, NEBR., KAN.
	Bushels			*Bushels*			*Bushels*			*Bushels*		
1866–1875	12.3	34.5	34.3	7.6	13.3	13.4	11.3	28.8	33.4	67.9	87.0	81.7
1876–1885	12.0	32.4	34.4	7.0	15.1	12.5	11.1	29.2	33.3	66.2	79.9	83.0
1886–1895	12.9	31.9	28.2	8.0	15.7	13.2	11.7	27.6	29.9	69.0	83.8	74.3
1896–1905	13.4	36.3	30.1	8.9	15.0	14.5	13.7	31.6	30.2	83.5	84.1	82.4
1906–1915	14.7	37.8	29.8	10.7	17.1	14.8	17.5	31.0	29.4	93.2	92.3	89.1
1916–1925	16.0	39.1	30.2	10.8	16.6	13.4	18.2	32.6	33.3	109.2	96.7	84.3
1926–1935	14.6	35.3	27.0	12.4	18.7	13.7	19.9	29.7	29.1	114.1	112.3	82.3
1936–1940[1]	15.2	40.8	28.6	12.4	19.2	12.6	20.9	31.3	32.5	103.4	117.4	86.8
1941–1945[1]	17.4	46.1	41.0	14.2	21.3	16.8	23.3	33.0	33.5	96.7	112.5	103.8
1936–1945	16.2	43.5	35.1	13.4	20.2	14.6	22.1	32.2	33.2	100.0	119.7	94.6

[1] Five years.

increase would be most marked in the case of corn and least marked in the case of wheat. The data in Table 1 show that this relationship actually holds true.

The average acre yields of cotton, tobacco, and potatoes by decades are shown in Table 2. These crops differ from those listed in Table 1 in that a very high proportion of the acreage devoted to each is heavily fertilized relative to the cereal crops. All show more or less continuous increases in crop yields throughout the period for which statistics are available. Table 2 also compares the average per acre yield of potatoes in Maine with the corresponding data for the country as a whole. In Maine the greater part of the potato crop is grown in one county, where more fertilizer is used than in any other similar area in the United States. The data in Table 2 show that the annual per acre yield of potatoes by decades increased 145 per cent in Maine during the past 80 years, whereas the corresponding increase for the country as a whole amounted to only 27 per cent. In so far as is known the factors, with one exception, that cause increased yields of potatoes in Maine are similar to those that bring about increased yields in other parts of the country. The one exception is the greater acre use of fertilizers in Maine. It seems logical to conclude therefore that although the increased yields that have taken place from decade to decade are due to many factors, the most effective are the extensive use of fertilizers and improvement in seed varieties.

Further evidence that crop yields can be increased by increasing the use of fertilizers is given in Table 3. In this table, crop yields of corn, wheat, oats, and potatoes grown over a period of years in a group of the South Atlantic States are compared by decades with the yields of the same crops in a group of North Atlantic States, and in a group of Midwestern States. The first group, Virginia, North Carolina, South Carolina, and Georgia, is representative of States that are large consumers of commercial fertilizers; the second group, New York, Pennsylvania, Ohio and Indiana, consists of States that are moderate consumers of fertilizers, while the States of the third group, Minnesota, Iowa, Nebraska, and Kansas, consumed very little fertilizer until recently. The crops listed in the table represent the principal crops common to the three groups of States and comprise an acreage equal to about 67 per cent of the total under cultivation in these States. Important crops, such as cotton and tobacco, were not used in this comparison of crop yields for the reason that they do not grow in all the States included in the three groups. A comparison of the yields of the same crops in the three groups of States for the first seven decades shows that the rates of increase from decade to decade during this period was greater for the first group of States than for the second, and greater for the second than for the third. In the third group of States where little or no fertilizer was used during this 70-year period, there did not seem to be any appreciable increase in crop yields from decade to decade.

Increased crop yields may be due, as already explained, to improved farm management and other technological advances, in addition to the use of fertilizers. The Southeastern States are the greatest consumers of fertilizers, but there is no evidence that such improvements have advanced further in these States than in other sections of the country. It would appear, therefore, that of the factors that may be instrumental in increasing crop growth in the Eastern part of the United States, the use of fertilizers is of greatest importance.

During the war years, or the second half of the last decade, the Midwestern States seem to have fallen in line with the country as a whole in showing a marked increase in yields of corn and certain other crops. Although the use of fertilizers in this area increased 4-fold during the war years, the total acre consumption is still relatively small. It is during this period that hybrid corn and other improved varieties of seed came into such general use. This development, coupled with a favorable rainfall during the entire five-year period, explains for the most part the bounteous harvests that we have enjoyed in recent years in so many parts of the country.

The total food production in the United States during the past 10 years was about 20 per cent greater than during the preceding 10 years. The percentage increase in the population during the same period amounted to only about 10 per cent. The present relationship between food supply and the population in the United States is very different from that prevailing in most countries and from what the English economist Malthus predicted in forecasts made about 150 years ago. In his book entitled "Principles of Population," Malthus advanced the view that the population was increasing in a geometric ratio while the produce of the soil could not be expected to increase by more than an arithmetic ratio. According to Malthus, if this relationship holds true, the population must eventually exceed the means of subsistence and the outcome must necessarily be followed by famine and misery, which in turn are likely to be accompanied by war, pestilence, and vice. These positive checks on the population will enable the means of subsistence to again catch up with the population. The latter in turn will again increase over the means of subsistence and the cycle will be repeated. While these predictions do not apply to the United States, as already explained, they do seem to be in accord with recent developments in China and India and with much of the history of the world.

NUTRITIVE VALUE OF CROPS

While the United States is favorably situated as regards the production of crops, there are those who claim that the nutritive value of the food and feed products grown in this country is decreasing. This assumed decrease in the quality of crops is attributed mainly to the depletion of our soils. Although the crops in certain local areas are known to be deficient

in one or more vitamins or mineral elements, there is no way of knowing at present whether the quality or nutritive value of our crops as a whole is decreasing or increasing. In a recent address, Dr. Truog, Head of the Department of Soils, University of Wisconsin, made a statement as follows:

"Absolutely no evidence exists to the effect that the judicious use of mineral fertilizer is at all injurious to soils, or tends to produce crops which are unsatisfactory as feed for animals or food for man. In fact evidence almost without end now exists showing clearly that the use of mineral fertilizers on depleted soils promotes the growth of crops which have superior nutritive values."

If this be assumed to be true then the nutritive value of crops should be increasing instead of decreasing as claimed by some, for the reason that the per acre consumption of fertilizers is increasing. It has been shown that while calcium and phosphorus deficiencies occur in animals grazing in certain parts of the country, such deficiencies do not occur in animals receiving forage grown on soils fertilized with superphosphate. It has also been demonstrated (1) that grass grown on fertilized soils is more palatable to farm animals than that grown on unfertilized soils; (2) that specific foods may be deficient in vitamins and minerals due to their removal or destruction in the processing to which the foods are subjected in their preparation for the market, rather than to any lack of fertility in the soil; and (3) that, while a deficiency of certain of the animal nutrient elements may occur in crops of maximum growth, this does not hold true in the case of the essential fertilizer elements.

Although much progress has already been made in the study of the nutritive value of crops, it must be admitted that much has still to be learned relative to this branch of agricultural science. It would be a development of inestimable value if a method of analysis could be found that would show for any given soil the fertilizer treatment required to give, under favorable weather conditions, a crop of maximum yield and nutritive value. Such a method has not yet been devised. Some soils are capable of producing crops of particularly fine flavor or quality but information is still lacking as to how other soils, similarly situated with respect to climate, should be treated to yield crops of corresponding high quality.

In a recent paper, Maynard of the U. S. Plant, Soil, and Nutrition Laboratory, called attention to the accomplishments that have been made in increasing crop yields by fertilization and other cultural practices; and in selecting varieties for appearance, for ability to withstand shipment to market, and for high resistance to disease, cold, and drought. These properties are all recognized as important, but it is held that nutritive value should come first. The subject is one that is now receiving a great deal of attention and it is reasonable to expect that advancement will continue in our knowledge of the relationship between the fertilization of the soil and the maximum yield and nutritive value of crops. Progress in

this field must necessarily be accompanied by the development of new and improved methods of analysis. More precise methods for diagnosing fertilizer needs are urgently needed. It is reasonable to expect that testing methods, which will indicate both the rate and kind of fertilizer most likely to yield maximum benefits for any given soil and crop, will in due course be developed. The time may thus come when improved methods of soil and fertilizer analysis, the objective responsible for the organization of our Association, may usher in a new era for better living and advancement.

ADDRESS: A WORD OF WELCOME

By T. J. WOOFTER, Director of Research, Federal Security Agency, Washington 25, D. C.

I am more than a little surprised that I have the temerity to address your Association. I am not an agricultural chemist—indeed, I am not a member of the chemical profession. I am, however, firmly convinced that the spirit of science is the same, no matter what the particular discipline or the technical method. The principal difference between scientific approach to human phenomena and to natural phenomena lies in the fact that the social scientists must express their results in terms of somewhat wider margins of probable error. It is both a pleasure and an obligation, on behalf of the Federal Security Agency, to extend to you the assurance that you are a welcome guest, or should I say a member of the family, at the fireside of an Agency devoted solely to the service of the public as a whole.

As Director of Research, I have a deep appreciation of the contribution which your Association has made and is making in ever-widening circles to the cause of consumer health, welfare, and well-being. One might even paraphrase the Scriptures and say: Without analytical methods, the people perish. Were it not for your tireless and disinterested studies, it would not be possible to adduce the unequivocal proof which is demanded by our courts that a food contains a dangerous amount of fumigant, that a tablet containing a dangerous drug carries several times the intended dosage, or that a cheese was made from unpasteurized milk of cows suffering from a disease transmissible to man as undulant fever. This is the essence of the logic of the inclusion of the Food and Drug Administration, along with the Office of Education, the Public Health Service, the Social Security Administration, and the Office of Vocational Rehabilitation, in the Federal Security Agency, for all of these organizations are primarily interested in the health and welfare of people as individuals.

If I may be permitted a little speculation, which is not subject to the same degree of strict proof which characterizes the methods in your Manual but which comes within the orbit of my own experience, I would

call attention to the tremendous saving from disease and death which has occurred in the past three or four decades. Fourteen years have been added to the life expectancy of infants, and predictions of mortality rates, based upon assumptions which, in 1922, seemed to be quite optimistic, were exceeded by 1935. I am, of course, aware that it would be unscientific to assign too great a weight to any one cause of such a multi-factored phenomenon, but I believe that you will never be accused of immodesty if it were asserted that the protective service which your preofession has built around the purity and potency of foods and drugs has played an appreciable part in this saving of life. There are some specific areas where the causal factor is more direct and apparent. The mortality rates from dysentery, diarrhea, and food poisoning have all declined by over two-thirds over their 1915 levels. In these fields the results of your efforts are more directly measurable.

Another reason why I am pleased to have you associated with the Federal Security Agency is your policy of insistence upon the usability of your methods by the profession as a whole and not just by a chosen few. With the present rapid increase in technical knowledge, the dissemination of the essential features of that knowledge becomes one of the most difficult and pressing problems. It is for that reason that I thoroughly approve of your policy of confining your *Book of Methods* to those procedures which may be generally applied by most chemists and of your efforts to make these methods widely available.

I realize, of course, that a goodly portion of the work of your Association has a strictly agricultural orientation, as it was in the beginning. Indeed, I believe that your precursor in 1880 was concerned solely with methods for phosphoric acid, nitrogen, and potash in fertilizers. But the world moves, and there soon came a time when Federal and State regulatory chemists became concerned with the health and welfare of folks, as well as of soils and farm animals. Your splendid *Book of Methods*, in its recent new edition, exemplifies this trend, for I find that more than two-thirds of its 900 pages relate directly to foods, drugs, cosmetics, and caustic poisons —that is to say, the protection of the health and the pocketbook of the American people as a whole. It is, therefore, altogether fitting and proper that you, serving as you do one of our favorite children, the Food and Drug Administration, should have the very warmest sort of welcome from the Agency which has given that Administration its sympathetic and wholehearted support. We have given your Washington Office shelter, and this is but a symbol of the support that you will find whenever and wherever you need it.

ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees, and collaborators in planning and developing their year's work. The remainder of the proceedings will follow in their usual order.

THIRD DAY

WEDNESDAY—AFTERNOON SESSION

REPORT OF THE EDITORIAL BOARD

By HENRY A. LEPPER, *Chairman*

The 6th Edition of *Methods of Analysis* has been in your hands for approximately a year. This edition marks a distinct advance in our publication, having grown, as it has, to 932 pages. It reflects the progress of the Association in the development of methods. That it has been enthusiastically received by the profession is demonstrated by the sale of over 5,000 copies in the first year. The remaining 4,000 copes are now being bound to fill orders which continue to be received. The task of preparing and editing the manuscripts, because of the size and increased scope, was the greatest ever performed, and the thanks and appreciation of the Association are to be heartily extended to the Editorial Committee on Methods of Analysis, of which Dr. H. J. Fisher is Chairman, and to the many referees and associate referees whose unselfish labors made the book possible.

The curtailment of our work because of the National emergency was reflected in a smaller volume of our *Journal*, for 1945. However, it is a notable addition to the record of progress which our *Journal* contributes to analytical chemistry each year. The interest of the profession in the *Journal* and its acceptance in this field are demonstrated by the increased subscription it has enjoyed.

The report of the Committee on the *Journal* will be presented by Dr. White, the Chairman, to be followed by the report of the Committee on Revision of Methods of Analysis, by Dr. Fisher.

Approved.

REPORT OF EDITORIAL COMMITTEE OF "THE JOURNAL"

W. B. WHITE, *Editor and Chairman*

Since there was no meeting last year, it seems in order to touch briefly on the history of the *Journal* during 1945. With the August issue Miss Katharine Ronsaville took over as Assistant Editor. By the end of the

year the ship was sailing under an entirely new working crew, and sailing very well indeed. For the first time our volume numbers appeared in Arabic, and the range in page numbers also appeared on the bound edge of each issue. Volume 28 ran to 828 pages, and it was not until the November issue that some degree of leanness began to be noticeable, and this has extended into Volume 29. The estimated number of pages in the November issue, now in press, brings the total for Volume 29 up to 448 pages. The contributed papers have, however, been excellent. Five appeared in February, 9 in May, 6 in August, and 8 will appear in November. Because there was no 1945 meeting, the reports of associate referees have come in rather irregularly, and this irregularity has naturally been reflected in the order in which we have had to print them. However, the last of them appeared in the August issue and we will start the 1947 volume with a clean slate.

Approved.

REPORT OF COMMITTEE ON REVISION OF METHODS OF ANALYSIS

The sixth edition of *Methods of Analysis* has been published and is already in your hands. The book itself constitutes our chief report, and little need be added here.

It is probable that very few if any books have ever been published that contained no errors. In the preparation of the present edition of *Methods of Analysis*, every paragraph of the manuscript and the two proofs was checked independently by at least three persons in an attempt to eliminate all errors. Nevertheless a few have been found in the printed copy, although none of those so far discovered has been very serious. We urgently request that each referee carefully examine his chapter and report any errors he finds to the Association so that notices of correction may be sent to subscribers.

The first edition of *Methods of Analysis* contained 417 pages; the sixth edition, with 932 pages, is over twice that size. The expansion of 175 pages over the fifth edition is much greater than any previous increase for a new edition. Entirely new chapters and chapters that existed in the fifth edition only by title are responsible for 76 of the new pages; the chapter on Extraneous Materials has 36 pages and the Vitamin chapter has 30.

Experience in the use of the book since its publication appears to confirm the convenience of the new decimal system of numbering that was adopted after some controversy. The decision of the Association to issue supplement sheets containing the methods adopted each year during the interval between editions will add greatly to the value of the book to subscribers and should tend to prevent errors in the use of the methods.

It should be unnecessary for us to state that without the faithful work

of many referees, associate referees, and collaborators this book could not have been prepared. We, the committee, know that if we had not been able to rely on the referees in preparing the manuscript and reading the proof, it would not have been the useful book that it is, and we wish at this time to express our thanks to all of them.

We submit this sixth edition and move its adoption.

HARRY J. FISHER, *Chairman*
L. E. WARREN
J. W. SALE
W. H. ROSS
W. F. REINDOLLAR
MARIAN LAPP OTIS

Approved.

No report was given by the Committee on Quartz Plate Standardization and Normal Weight, nor by the Committee to Confer with American Public Health Association on Standard Methods of Milk Analysis.

REPORT OF COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS

By L. S. WALKER, *Chairman*
Official First Action

GUARANTEEING IN TERMS OF ELEMENTS

All fertilizer components with the exception of potash (K_2O) and phosphoric acid (P_2O_5), if guaranteed, shall be stated in terms of the elements.

Fused tricalcium phosphate is a product composed chiefly of the alpha form of the compound represented by the formula $Ca_3(PO_4)_2$. Its fineness and content of available phosphoric acid (P_2O_5) shall be stipulated. Example: Fused tricalcium phosphate—twenty-five per cent (25%) available phosphoric acid (P_2O_5).

Calcium metaphosphate is a product composed chiefly of the vitreous compound indicated by the formula $Ca(PO_3)_2$. Its fineness and its content of available phosphoric acid (P_2O_5) shall be stipulated. Example: Calcium metaphosphate—sixty per cent (60%) available phosphoric acid (P_2O_5).

Potassium metaphosphate is a product composed chiefly of the crystalline compound represented by the formula KPO_3. Its fineness and content of phosphoric acid (P_2O_5) and of potash (K_2O) shall be stipulated. Example: Potassium metaphosphate—fifty-five per cent (55%) available phosphoric acid (P_2O_5): thirty-seven per cent (37%) potash (K_2O).

Double sulfate of potash and magnesia (Langbeinite). Double sulfate of potash and magnesia (Langbeinite) is a commercial product containing not less than twenty-one per cent (21%) of potash (K_2O), nor less than fifty-three per cent (53%) of sulfate of magnesia and not more than two and one-half per cent (2.5%) of chlorine.

PROPOSED DEFINITIONS

Fused Calcium—Magnesium Phosphate is a product derived from the fusion of rock phosphate with approximately thirty per cent (31%) of magnesium oxide

(MgO), as such as a mineral silicate. Its fineness and content of available phosphoric acid (P_2O_5) shall be stipulated. Example: Fused calcium—magnesium phosphate, twenty per cent (20%) available phosphoric acid (P_2O_5).

Approved.

REPORT OF COMMITTEE ON RECOMMENDATIONS OF REFEREES

W. F. REINDOLLAR, *Chairman*

A number of factors arising from the War have combined to interfere seriously with the work of our Association. Among these may be cited the need for manpower by military agencies and war industries which caused the loss of many collaborators and referees, the shortage of necessary chemicals, reagents, and laboratory apparatus, and finally, the limitations on travel which prevented the holding of the 1945 meeting. In the face of these handicaps, however, and in spite of the pressure of increased routine activities, many chemists have found some time to devote to the development of analytical methods, and it is the reports of these studies which constitute our present program.

The Association of Official Agricultural Chemists exists primarily for the purpose of securing and devising uniform and accurate methods of analyses for those products coming within its sphere of interest. Its continued success, therefore, depends in a large measure upon the extent to which it can attain these objectives, and this, in turn, depends upon the number of qualified chemists who are willing to assist in one or more of the numerous projects. The need for securing referees, associate referees, and collaborators cannot be too strongly stressed at the present time. Any analyst who has profited in any way through the application of an official or tentative method in his own work is, in turn, obligated to reciprocate by contributing some of his time and talent to the Association that made these methods available. The need was never greater than now.

The chairman extends his sincere appreciation to the members of the several subcommittees, referees, associate referees, collaborators, and all others whose labors have made possible those gains which occurred during the difficult war years.

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES*

By E. L. GRIFFIN (Insecticide Division, Livestock Branch, P. M. A., U. S. Department of Agriculture, Washington, D. C.), *Chairman;* L. S. WALKER,† and H. A. HALVORSON.

FEEDING STUFFS

It is recommended—

(1) That the following official (first action) methods be made official (final action):

Sampling, 27.1, page 404.
Water-soluble acidity, 27.41, page 414.
Rice hulls in rice bran, 27.43, page 414.
Oat hulls in oats and oat feeds, 27.44, page 414.

(2) That an Associate Referee be appointed to continue work on the method for hydrocyanic acid formed by hydrolysis of glucosides in feeding stuffs.

(3) That the two tentative methods for iodine in mineral mixed feeds (**27.54–27.57,** pp. 417–418) be checked by the Associate Referee on Mineral Mixed Feed with the method discussed by Johnson and Frederick (*This Journal,* **23,** p. 688, 1940).

(4) That the work on lactose in mixed feeds be continued.

(5) That the determination of fat in fish meal be further studied.

(6) That further work be done on the detection of adulterants in condensed milk by-products.

(7) That further work be done on fat in cooked animal feeds containing cereals.

(8) That further work be done on the determination of crude fat or ether extract in feeds.

(9) That work on the activity of yeast, microscopic examination, fluorine, mineral constituents of mixed feed, crude fibre, and protein evaluation in fish and animal products be continued.

(10) That an Associate Referee be appointed to study the methods for crude protein with special reference to catalysts.

(11) That collaborative work on the sampling and analysis of condensed buttermilk be continued.

FERTILIZERS

It is recommended—

(1) That the study of methods for calcium in fertilizers be discontinued, that the title of the Associate Referee on Calcium and Sulfur be changed to Associate Referee on Sulfur and that the work on sulfur be continued.

* These recommendations, submitted by Subcommittee A, were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis, A.O.A.C.,* 1945.

† Served for G. E. Grattan.

(2) That in view of the fact that the method for potash (**2.40–2.42,** pp. 31–32) involves the use of ammonium oxalate and diglycol stearate solution, further consideration be given to its advisability before changing the heading to "Water-soluble Potash."

(3) That the method for the recovery of platinum, including the preparation of platinum solution (*This Journal,* **28,** 782–783, 1945), be adopted as tentative.

(4) That a study be made of the use of mechanical shakers in the ammonium citrate method for available phosphoric acid, with the object of making modifications in the methods for determining water-soluble and citrate-insoluble phosphoric acid, if desirable.

(5) That a study be made of the applicability of the ammonium citrate method to basic slag, with the object of adopting it in place of the citric acid method if the change is found desirable.

(6) That further collaborative work be done on methods of determining nitrogen in ammonium nitrate with special reference to the Devarda method and the use of various connecting traps and bulbs.

(7) That an analysis be made of the published results of plant-growth experiments and tests with the various types of alpha phosphates (sintered, fused, and calcined products) in order that a better understanding may be had of the fertilizing values of such products in comparison with those of superphosphate and other sources of P_2O_5.

(8) That further study be made of the nature of the citrate-insoluble components of sintered, fused, and calcined alpha phosphates.

(9) That a study be made of the aging of the molybdate solution used in the volumetric method for P_2O_5 to see if a time limit should be put on its use or an addition made to preserve it.

(10) That the Associate Referee on magnesium and manganese continue his work.

(11) That work on the determination of potash be continued as recommended by the Associate Referee.

(12) That work on acid- and base-forming quality, boron, copper, and zinc, and moisture be continued.

(13) That the term "80% alcohol" in the method for potash (**2.40–2.42,** pp. 31–32) be clarified by making the editorial change to "80% ethyl alcohol by volume."

(14) That the determination of total nitrogen in mixed fertilizers by the modified Kjeldahl-Gunning method be studied in reference to the loss of nitrogen with fertilizers high in chlorides and nitrates.

(15) That methods of sampling be studied.

INSECTICIDES AND FUNGICIDES

It is recommended—

(1) That the method for preparation of pure rotenone reagent, as described by the Referee, be added to sec. **6.110,** pp. 74–76, on Rotenone.

(2) That the lead chlorofluoride method (**6.18–6.19,** pp. 58–60) for the determination of fluorine be adopted as official (final action).

(3) That the method for phosphide phosphorus in rodenticides be further studied.

(4) That methods for the analysis of rodenticides containing "1080" (sodium fluoroacetate) and Antu (alpha naphthyl thiourea) be studied.

(5) That work on the determination of nicotine and nornicotine be continued with attention being centered on the apparatus.

(6) That the methods numbered **2** through **6,** as given by the Associate Referee on DDT, be adopted as tentative methods for the determination of DDT in the absence of other organic chlorine-containing compounds.

(7) That Method 1, as given by the Associate Referee on DDT, with the revised sampling procedure, be adopted as a tentative method and that it be subjected to further collaborative study.

DISINFECTANTS

It is recommended that work on this subject be continued.

LEATHERS AND TANNING MATERIALS

No report was received. It is recommended that this work be continued.

PLANTS

It is recommended—

(1) That the work on carotene in plant tissue be continued.

(2) That the tentative methods for determining chlorophyll in plant tissue be made official (first action).

(3) That the tentative o-phenanthroline colorimetric method for iron in plants be made official (first action).

(4) That the study of methods for zinc in plants be continued.

(5) That the work of the Associate Referees on sampling, iodine and boron, carbohydrates, and copper and cobalt be continued.

SOILS AND LIMING MATERIALS

It is recommended—

(1) That the "combination dithizone-spectrographic method" and the polarographic procedure for the determination of zinc in soils be studied further.

(2) That the determination of copper in soils be studied.

(3) That the utilization of carmin as an indicator in the determination of the boron content of soils be studied further.

(4) That further studies of hydrogen ion concentration in soils of the arid and semi-arid regions be based upon soil systems of moisture content representative of air-dry soil.

(5) That the analytical technique previously proposed for ignition of charge and distillation of fluorine therefrom be studied collaboratively.

(6) That the "2-point" titration procedure for the determination of exchangeable H in soils be studied further in relation to liming practice.

(7) That a survey and comparisons be made of methods for the de-

termination of phosphorus **(a)** that fraction in "available" state and **(b)** the proportions of organic-inorganic forms therein.

(8) That a survey and comparisons be made of methods for the determination of exchangeable K in soils.

(9) That the direct titration against bromocresol green (Method 2) be adopted as optional for the determination of the neutralization value of blast furnace slags.

(10) That the tentative procedures be annotated by the statement "without correction for sulfide content."

(11) That correction for sulfide sulfur be studied further.

STANDARD SOLUTIONS

It is recommended—

(1) That the preparation and standardization of potassium dichromate solutions be studied.

(2) That study of sodium thiosulfate solutions be dropped.

(3) That the studies on buffer solutions be continued.

(4) That the method for preparation and standardization of thiocyanate solutions, as presented by the Associate Referee, be adopted as official (first action) and that the work be continued with a view to its adoption (final action).

(5) That studies on potassium permanganate solutions be continued.

(6) That the studies on bromide-bromate solutions be continued.

(7) That the methods for standardization of titanium trichloride solutions be further studied.

VITAMINS

It is recommended—

(1) That the microbiological method for the determination of riboflavin, described in *This Journal*, **29**, 25 (1946) be adopted as tentative and that the method **36.32–36.40**, inclusive (pp. 613–617) be dropped.

(2) That the fluorometric method for the determination of riboflavin described in the report of the Associate Referee be studied collaboratively during the coming year.

(3) That the 1945 U.S.P.-A.O.A.C. method for nicotinic acid, as described by the Associate Referee, be made official (first action).

(4) That absorbents be further studied in order to develop tests by which suitability of an absorbent for the determination of carotene can be ascertained.

(5) That the methods for carotene, **36.7–36.15** inclusive (pp. 600–606) be dropped and that the method described in *This Journal*, **29**, 21 (1946) for the determination of carotene be adopted as tentative.

(6) That the work of the Associate Referees on vitamins A, B_1, B_2, C, and D, nicotinic acid, pantothenic acid, and carotene be continued.

(7) That an Associate Referee be assigned the topic, folic acid, for study and investigation.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES*

By F. H. WILEY (U. S. Food and Drug Administration, Washington, D. C.), *Chairman;* H. J. FISHER, and G. R. CLARK.

NAVAL STORES

No report was received from the referee, and it is recommended that the subject be continued.

RADIOACTIVITY

No reports were received. It is recommended—

(1) That studies involving the quantum counter be continued.

(2) That analysis by radon measurement and alpha particle counter be continued.

SPECTROGRAPHIC METHODS

No report was received. It is recommended that the subject be continued.

VEGETABLE DRUGS AND THEIR DERIVATIVES

It is recommended—

(1) That the study of methods for determining physostigmine in ointments be continued and reassigned.

(2) That the method submitted by the Associate Referee for quinine ethyl carbonate be adopted as tentative and the subject closed.

(3) That the investigation on the separation of theobromine and phenobarbital be continued.

(4) That the tentative method for the determination of prostigmine be adopted official (first action) and the subject closed.

(5) That the study of the separation of aminopyrine, ephedrine, and phenobarbital be continued.

(6) That the work on ephedrine be continued and the method reported by the Associate Referee be submitted for collaborative study.

(7) That the study of methods for the determination of camphor in spirits of camphor be continued.

(8) That a study be made on chemical methods for the estimation of penicillin.

(9) That studies be continued on chemical methods for ergot alkaloids.

(10) That studies on methods for determination of quinine be continued.

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* These recommendations submitted by Subcommittee B were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis*, A.O.A.C., 1945.

SYNTHETIC DRUGS

It is recommended—

(1) That the study of methods for determining phenothiazine be discontinued.

(2) That the method for the determination of plasmochine reported by the Associate Referee be adopted as tentative and the subject closed.

(3) That Methods I and II previously reported for the determination of 8-hydroxyquinoline sulfate be adopted tentatively and the subject closed.

(4) That the study of methods for methylene blue be continued and include spectrophotometric procedures.

(5) That Methods I and III previously reported for the determination of metrazol be tentatively adopted and that the topic be closed.

(6) That the study of methods for the determination of sulfanilamide derivatives be continued.

(7) That the method reported by the Associate Referee for the determination of phenolphthalein in bile salts be adopted tentatively and that the topic be closed.

(8) That the development of a method for the estimation of atabrine be continued.

(9) That the study of the method previously reported for the determination of demerol be continued.

(10) That the development of a method for propadrine hydrochloride be continued.

(11) That the study of methods for determining carbromal be continued and reassigned.

(12) That the methods for determination of dihydrocodeinone be further investigated.

(13) That the study of methods for butacaine sulfate be continued and reassigned.

(14) That the application of spectrophotometric methods to drug analysis be further studied.

(15) That a study of methods for the determination of trichloroethylene be undertaken.

(16) That a study of methods for thiouracil be undertaken.

MISCELLANEOUS DRUGS

It is recommended—

(1) That the development of microchemical tests for alkaloids and synthetics be continued.

(2) That the study of the ethanolamine method for mercurials be continued and expanded to include phenyl mercuric acetate and potassium mercuric iodide and that the subject be reassigned.

(3) That the development of a method for the separation of bromides, chlorides, and iodides be continued.

(4) That the study of methods for the determination of organic iodides be continued.

(5) That the study of the analysis of compound ointment of benzoic acid be continued.

(6) That the development of methods for alkali metals be continued and reassigned.

(7) That the work reported by the Associate Referee on the determination of glycols and related compounds be continued.

(8) That the studies reported by the Associate Referee on preservatives and bacteriostatic agents in ampul solutions be continued.

(9) That the determination of phosphorus, calcium, and iron in vitamin preparations be further studied and reassigned.

(10) That the study of the analysis of effervescent antipyrine with caffeine be discontinued.

(11) That the official method for iodine (*Book of Methods*, 6th edition, **39.202**) be reinvestigated.

(12) That the use of superheated steam in the separation of drugs be investigated.

(13) That chemical methods for the estimation of estrone and estradiol be studied.

(14) That methods for chromatographic separation of drugs be studied.

DRUG BIOASSAYS

It is recommended—

(1) That the study of enteric coatings be continued.

(2) That the bioassay methods for ergometrine (ergonovine) be further studied.

(3) That the study of bioassay methods for digitalis preparations be continued.

COSMETICS AND COAL-TAR COLORS

(1) That the study of alkali in cuticle removers be continued and reassigned.

(2) That the study of the analysis of cosmetic creams be continued.

(3) That the methods of analysis of cosmetic powders be further studied.

(4) That the study of cosmetic skin lotions be continued.

(5) That the methods for analysis of deodorants and anti-perspirants be continued.

(6) That methods for analysis of depilatories be further investigated and the subject be reassigned.

(7) That the study of the analysis of hair dyes and rinses be continued.

(8) That the study of methods of analysis for hair straighteners be continued.

(9) That the study reported by the Associate Referee on mascara, eyebrow pencils, and eye shadow be continued.

(10) That the determination of mercury salts in cosmetics be further investigated.

(11) That a further study be made of methods for determining moisture in cosmetics.

(12) That the method reported by the Associate Referee for pyrogallol in hair dyes be adopted tentatively and that the study be continued to eliminate the interference of henna on the results of the assay.

(13) That the method for resorcinol in liquid hair dyes reported by the Associate Referee be officially adopted (first action) and the subject closed.

(14) That the study of urea in deodorants be discontinued.

(15) That the determination of acetates, carbonates, halides, and sulfates in certified coal-tar colors be further studied.

(16) That the study on effects of buffers and solvents in titanium trichloride titrations be continued and reassigned.

(17) That the ether extract in coal-tar colors be further studied.

(18) That the methods for halogens in halogenated fluoresceins be further studied and the topic reassigned.

(19) That the topic on Intermediates in certified coal-tar colors be divided into six projects as follows:

(a) Volatile amine intermediates in certified coal-tar colors.
(b) Nonvolatile unsulfonated amine intermediates in certified coal-tar colors.
(c) Sulfonated amine intermediates in certified coal-tar colors.
(d) Unsulfonated phenolic intermediates in certified coal-tar colors.
(e) Sulfonated phenolic intermediates in certified coal-tar colors.
(f) Intermediates derived from phthalic acid in certified coal-tar colors.

(20) That the study of mixtures of coal-tar colors for drug and cosmetic use be continued.

(21) That the methods for analysis of lakes and pigments be further investigated.

(22) That the study of spectrophotometric testing of coal-tar colors be continued.

(23) That the study of subsidiary dyes in drug and cosmetic colors be continued.

(24) That a study of methods for the determination of lead in coal-tar colors be undertaken.

(25) That study of methods for identification of certified coal-tar dyes be continued.

(26) That studies on hair straighteners be continued.

(27) That studies on nail cosmetics be discontinued for the present.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES*

By J. O. CLARKE (U. S. Food and Drug Administration, Chicago, Ill.), *Chairman;* C. S. LADD, and JOSEPH CALLAWAY.

PROCESSED VEGETABLE PRODUCTS

It is recommended—

(1) That studies of methods for quality and fill of container be continued.

(2) That studies be continued on determination of moisture in dried vegetables.

(3) That the method for determination of catalase in frozen vegetables recommended by Dr. Lineweaver be adopted as tentative.

FILL OF CONTAINER METHODS

It is recommended that studies be continued on methods of fill of container of foods, drugs, and cosmetics.

COFFEE AND TEA

It is recommended—

(1) That the phrase "Not applicable to coffee extracts" be inserted below the title of the Power-Chesnut method **(18.14).** (First action).

(2) That the Fendler-Stüber method (modified) **(18.15)** be made official (first action).

(3) That the Bailey-Andrew method for caffeine in tea **(18.41)** be adopted as an official method (first action) for caffeine in coffee.

(4) That the study of methods for the determination of chlorogenic acid in coffee be continued.

COLORING MATTERS IN FOODS

It is recommended—

(1) That the method for the detection of small (or large) amounts of Tartrazine FD&C Yellow No. 5 in alimentary paste be submitted to further collaboration.

(2) That investigational work be continued on the quantitative separation and estimation of FD&C Yellow No. 5 (Tartrazine) and FD&C Yellow No. 6 (Sunset Yellow F.C.F.).

(3) That investigational work be undertaken to separate and determine quantitatively FD&C Green No. 2 (Light Green S.F. yellowish), FD&C Green No. 3 (Fast Green F.C.F.), and FD&C Blue No. 1 (Brilliant Blue F.C.F.).

(4) That investigational work be undertaken to separate and estimate quantitatively FD&C Yellow No. 3 (Yellow A.B.), FD&C Yellow No. 4

* These recommendations submitted by Subcommittee C were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis, A.O.A.C.*, 1945.

(Yellow O.B.), FD&C Orange No. 2 (Orange S.S.), and FD&C Red. No. 32 (Oil Red X.O.).

(5) That collaborative work on analytical methods for coal-tar colors certifiable for use in foods be conducted. This general subject parallels to some degree studies in Coal-Tar Colors being pursued under Committee B. Committee C suggests that the Chairman of the Committee on Recommendations of Referees assign the subject to prevent duplication.

DAIRY PRODUCTS

It is recommended—

(1) That there be appointed an associate referee on the phosphatase test as an index of pasteurization, and that he study the development of a unified method applicable to milk and other dairy products.

(2) That methods for the detection of reconstituted milk be studied.

(3) That studies be continued on a quantitative method for gelatin and other stabilizers in frozen desserts.

(4) That studies be continued on chlorine in milk.

(5) That studies on methods for fat, moisture, and sampling of cheese be continued.

(6) That studies of methods for acidity of milk be continued.

(7) That studies be continued on methods for the preparation of samples of butter.

(8) That the tentative method for ash in milk **(22.16)** be further studied with a view to adoption as official.

(9) That the official first action method **(22.146 b)** for the preparation of samples of frozen desserts be further studied.

(10) That an associate referee be appointed to study methods for ice cream and frozen desserts.

(11) That the method for sour serum test be investigated.

EGGS AND EGG PRODUCTS

It is recommended—

(1) That studies be conducted on methods for the determination of added glycerol.

(2) That the methods for the determination of acidity of fat be studied.

MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN FOODS AND DRUGS

It is recommended—

(1) That the tentative methods described by the Referee and Associate Referees be changed, as recommended by the Referee and Associate Referees in *This Journal*, **29**, p. 54–65, and in current reports.

(2) That the studies on methods for extraneous matter in foods and drugs be continued.

(3) That the Referee study the nomenclature used in all methods.

DECOMPOSITION IN FOODS

It is recommended—

(1) That further studies be made of chemical criteria of decomposition.

GELATIN, DESSERT PREPARATIONS, AND MIXES

It is recommended—

(1) That methods for the determination of jelly strength in gelatin be studied, bearing in mind the method of the Edible Gelatin Manufacturers Research Association of America.

FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That collaborative study be continued on the Modified Rose-Gottlieb Method for determination of ether extract in fish.

(2) That the several methods for determining total solids and ether extract (*This Journal*, **26**, 226–232) be further studied collaboratively with a view to selecting the most suitable method for each constituent.

GUMS IN FOODS

It is recommended—

(1) That the method for detection of gums in cheese be further studied:

(a) in its quantitative application
(b) in its application to the detection of soluble alginates.

(2) That the method for the detection of gums in starchy food, *This Journal*, **29**, 250, be further studied collaboratively.

(3) That studies be continued on the detection of gums in cacao products.

(4) That an associate referee be appointed to undertake studies on the detection of gum in pectin-containing food products.

(5) That studies for the detection of gums in frozen desserts be continued.

(6) That gums in mayonnaise and French dressing be studied.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That the studies of methods for the determination of soybean flour and dried skim milk in meat be continued.

METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

It is recommended—

(1) That methods for cadmium be further studied.

(2) That methods for copper be further studied.

(3) That methods for DDT be further studied.

(4) That methods for the determination of mercury be further studied.

(5) That methods for the determination of zinc be further studied.

MICROBIOLOGICAL METHODS

It is recommended—

(1) That studies be continued on methods for the determination of liquid, dried, and frozen eggs, including methods for microscopic count.

(2) That studies be continued on methods for the examination of canned fish, canned meats, canned tomatoes and other acid vegetables and fruit products, nuts and nut products, and frozen fruits and vegetables, including collaborative work if possible.

MICROCHEMICAL METHODS

It is recommended—

(1) That study on microchemical methods be continued.

NUTS AND NUT PRODUCTS

(1) That studies on nuts and nut products be continued.

OILS, FATS, AND WAXES

It is recommended—

(1) That the tentative S.P.A. method **31.40** be adopted as an official method (first action).

(2) That the chromatographic procedure for the purification of the unsaponifiable matter be studied.

(3) That the official F.A.C. method for unsaponifiable matter **31.37–31.39,** inclusive, be deleted (first action).

(4) That the tentative method for squalene **31.41–31.43,** inclusive, be adopted as official (first action).

(5) That studies on methods for the determination of the stability of fats be continued.

(6) That studies on methods for the estimation of peanut oil be continued.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

It is recommended—

(1) That work be continued on benzoates and esters of benzoic acid.

(2) That the determination of saccharin in baked goods be studied.

(3) That work be initiated for the detection and/or determination of antioxidants in fats and the subject assigned to the section on oils.

(4) That studies be undertaken on methods for the detection and/or determination of quaternary ammonium compounds, mold inhibitors—propionates, diacetates, thiourea, and dichloracetic acid.

(5) That the subject of sulfites in foods be dropped for the present.

(6) That following **32.30** in the chapter, which is the qualitative test for fluorides, the words "quantitative, see **29.22** through **29.33**" be inserted.

(7) That a referee be appointed to continue the work on qualitative tests for formaldehyde.

(8) That methods for the detection and identification of monochloracetic acid be further studied.

SPICES AND CONDIMENTS

It is recommended—

(1) That the quantitative method of Mallory and Love for caramel in vinegar be studied before further collaborative work is done on the tentative method for detection of caramel.

(2) That the tentative permanganate oxidation number be studied collaboratively.

(3) That the tentative qualitative **(33.85** and **33.86)** and the quantitative **(33.87)** methods for free mineral acids be dropped.

(4) That the methods for the quantitative determination of free mineral acids be further studied.

(5) That work on determination of tartrates in vinegar be continued.

(6) That the method for starch in salad dressings (*This Journal*, **27**, p. 260) be adopted as tentative, and that work on the method be continued.

(7) That the tentative method for starch in prepared mustard be modified in accordance with the improvements described in the Associate Referee's report; that it be made tentative for mustard flour, and that study be continued.

(8) That studies be made of a suitable method for determination of ash in prepared mustard.

(9) That the official method for copper-reducing substances by direct inversion **(33.40)** be dropped (First action).

(10) That studies be made of a suitable method for determination of sugars in prepared mustard.

(11) That studies be continued on volatile oil in spices.

REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES*

By J. WALTER SALE (U. S. Food and Drug Administration, Washington, D. C.), *Chairman;* KENNETH L. MILSTEAD, and C. S. FERGUSON.

ALCOHOLIC BEVERAGES

Malt Beverages, Brewing Materials, and Allied Products:

It is recommended—

(1) That the following tentative methods for malt be adopted as official, first action: Sampling **(14.39)**; Preparation of sample **(14.40)**; Bushel

* These recommendations of Subcommittee D were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis, A.O.A.C.*, 1945.

weight **(14.41)**; 1,000 Kernel weight **(14.45)**; Moisture in caramel malt and black malt **(14.52)**; Diastatic power **(14.61)**.

(2) That the following official (first action) methods for malt be adopted as official, final action: Moisture **14.49–14.51,** inclusive; Color of wort **(14.60)**.

(3) That a method for determination of essential oil in hops be studied.

(4) That the following tentative methods for beer be adopted as official, first action: Extract of original wort **(14.7)**; Real degree of fermentation or real attenuation **(14.8)**; Apparent degree of fermentation or apparent attenuation **(14.9)**; Total acidity—Indicator Titration method **(14.10)**; Total Acidity—potentiometric titration method **(14.11)**; H-ion Concentration (*p*H)—Electrometric method **(14.12)**.

(5) That the following tentative methods for hops be adopted as official, first action: Sampling **(14.80)**; Physical examination **(14.81)**; Preparation of sample for chemical analysis **(14.82)**; Moisture **(14.83)**; Resins **(14.84–14.89,** inclusive).

(6) That the following tentative methods for brewing sugars and syrups be adopted as official, first action: Extract **(14.90)**; Non-extract (apparent water) **(14.91)**; Fermentable extract— **(b)** Rapid Fermentation method **(14.92)**; Protein **(14.93)**; Iodine reaction for unconverted starch **(14.95)**; Acidity **(14.96)**; H-ion concentration (*p*H) **(14.97)**; Ash **(14.98)**; Total reducing sugars—Munson-Walker general method **(14.99)**.

(7) That the following tentative methods for wort be adopted as official, first action: Specific gravity **(14.103)**; Original extract or original gravity **(14.104)**; Fermentable extract **(14.105)**; Iodine reaction **(14.106)**; Total acidity **(14.107)**; H-ion concentration (*p*H) **(14.108)**; Color **(14.109)**; Protein **(14.110)**; Total reducing sugars **(14.111)**.

(8) That the following official (first action) methods for beer be adopted as official, final action: Apparent extract or saccharimetric indication **(14.4)**; Iodine reaction for unconverted starch **(a)** For light beer **(14.27)**.

(9) That the following official, first action, methods for cereal adjuncts be adopted as official, final action: Sampling **(14.65)**; Preparation of sample **(14.66)**; Physical characteristics **(14.67)**; Moisture **(14.68, 14.69)**; Oil or petroleum benzine extract **(14.70, 14.71)**; Extract **(14.72–14.75,** inclusive); Crude fat or ether extract **(14.76)**; Protein **(14.77)**; Ash **(14.78)**; and crude fiber **(14.79)**.

(10) That the method prepared by the Associate Referee for color in beer be adopted as tentative, and that the study on photoelectric beer color evaluation as well as work on beer turbidity methods be continued.

(11) That the tentative manometric method for CO_2 in beer be further studied with a view to including a measurement for head-space air.

(12) That study of the method for the determination of carbon dioxide in beer be continued.

(13) That study of methods for detection of inorganic elements in beer

be continued in cooperation with the Referee on Metals in Foods and the Associate Referee on Iron in Cereals.

(14) That methods for testing soluble starches used in diastatic power determination of malt be studied.

(15) That methods for determination of total solids and yeast solids in yeast be studied.

(16) That studies on methods for cereal adjuncts be discontinued for the present.

Wines:

It is recommended—

(1) That study of the spectrophotometric examination of wines be continued.

(2) That the shorter method for caramel determination, known as Mathers method, be studied collaboratively.

(3) That chromatographic studies in wines be continued.

(4) That study of methods for methanol in wines and distilled liquors be continued.

Distilled Liquors:

It is recommended—

(1) That the effect of alcoholic content on *p*H of distilled liquors be discontinued.

(2) That the obscuration method for determining the true proof of blended spirits be studied.

(3) That methods of analysis with reference to the aging or maturing of whisky in laminated (plywood) barrels be studied.

(4) That the methods for total **(16.10)** and fixed acids **(16.12)** be made official, final action.

(5) That studies in formal titrations be discontinued.

(6) That study of cordials and liqueurs be continued.

CACAO BEAN AND ITS PRODUCTS

It is recommended—

(1) That the work on lecithin (*This Journal*, **25**, 717) be continued.

(2) That the work on the determination of maltose and of lactose in the presence of other reducing sugars be continued.

(3) That methods for the determination of cacao ingredients be further studied.

(4) That work on the determination of fat in beverage bases and other refractory material be continued.

(5) That line 7 of the third paragraph under **19.16** "ca (20° below 25°)" be changed to "20° or lower."

(6) That collaborative work on the tentative method for pectic acid, **19.16,** be continued.

CEREAL FOODS

It is recommended—

(1) That an Associate Referee be appointed to study the application of methods for the determination of phosphorus in cereal products.

(2) That "wheat" appear in the list of other grains in the title preceding **20.70.**

(3) That the method **20.8** for "Original ash of flour in phosphated and self-rising flour" be made official, final action.

(4) That the dry ashing method for iron **20.9–20.12,** inclusive, be made official, final action, with respect to flour and bread, and official, first action, with respect to macaroni products, degerminated corn meal and whole corn meal, applicable to enriched products and studies be continued on corn meal and macaroni products.

(5) That the wet ashing method for iron (*This Journal,* **29,** 275) be made official, first action, and that study of it be discontinued.

(6) That the method for calcium, **20.13,** be made official, final action.

(7) That work on rye flour in rye bread and in flour mixtures be discontinued.

(8) That the decantation method for determination of H-ion, **20.27,** be adopted as official, final action.

(9) That studies on determination of starch in raw and cooked cereals be continued.

(10) That the tentative method for determination of fat acidity in grain, flour, cornmeal, and whole wheat flour **(20.18–20.21,** inclusive) be further studied and also that the relationship of acidity to unsoundness be studied.

(11) That a new Associate Referee be appointed to study the application of the method for reducing and non-reducing sugars in flour, **20.29,** to the determination of sugars in bread and other cereal products.

(12) That the study of the tentative method for the determination of benzoyl peroxide in flour, **20.53,** be continued.

(13) That work be continued on methods for determination of available carbon dioxide in self-rising flour containing added calcium carbonate.

(14) That the method for the determination of lactose in bread (*This Journal,* **24,** 630) be further studied.

(15) That the determination of milk fat in bread, **20.86,** be further studied.

(16) That the semi-autolytic method for proteolytic activity of flour (*This Journal,* **29,** 258) be discontinued, and that other methods be developed.

(17) That the study of the methods for the determination of moisture, ash, nitrogen, crude fiber, and ether extract in soy bean flour and other soy bean products be continued.

(18) That studies be made on the detection and determination of soy

bean flour cereal products by immunological methods or suitable means of estimation.

(19) That the studies of the determination of added inorganic materials in phosphated and self-rising flour be continued.

(20) That the method referred to in *This Journal*, **25**, 83–84, for the determination of unsaponifiable matter and sterols in noodles be studied to determine their applicablity to other foods containing eggs.

(21) That studies of methods for the determination of albumin in noodles and other farinaceous egg-containing products be continued.

(22) That the methods for the determination of moisture and fat by acid hydrolysis in fig bars and raisin-filled crackers, **20.106** and **20.107**, respectively, be adopted as official.

(23) That the tentative method for the determination of total solids **20.84 (b)** for raisin bread and bread containing raisins and fruits be adopted as official, first action, except that the air-dry sample be dried in an uncovered dish in vacuum oven for about 16 hours at 70°C. under pressure not to exceed 50 mm of Hg and that the study on the determination of moisture, fat, crude fiber, ash, and protein in bakery products be continued.

(24) That the study on the determination of moisture in all flour-like products containing sodium bicarbonate as one of its constituents be continued.

(25) That the study of methods for the determination of bromates in flour be continued.

(26) That the determination of apparent viscosity measurements of flour be continued.

BAKING POWDER

It is recommended—

(1) That additional work be done on various types of baking powders, using the gasometric method, **17.4–17.6**, inclusive and **17.8**, for the determination of residual carbon dioxide, with special attention to the time and method of heating in preparation of the residual solution.

(2) That an investigation be made on modifying the present gravimetric method, **17.2**, **17.3**, and **17.7**, by changing from the sulfuric and potassium hydroxide absorption bulbs to the use of "caroxite" or "ascarite and anhydrone."

(3) That a comparison be made of the official gasometric method, **17.6**, with a method similar in principle to that given in Quartermaster Corps Tentative Spec. for Baking Powder—C.Q.D. No. 326, May 24, 1946, which in brief involves a correction on the unknown samples determined by analysis of a known sample under the same conditions.

(4) That the method (**17.4–17.6**, inclusive and **17.8**) be adopted as tentative for baking powder containing added calcium carbonate with the al-

ternate use of HCl (1+2) for H_2SO_4 (1+5), and the method **17.9** be made tentative for baking powders containing added calcium carbonate.

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended:—

(1) That the collaborative study of the reflux method for determination of peel oil in citrus fruit juices and the use of the modified oil separation trap be continued.

(2) That collaborative work be continued on the method for determination of beta-ionone where small amounts are present.

(3) That collaborative studies of the Ripper method for determination of aldehydes in spirits as applied to lemon oils and extracts be continued.

(4) That collaborative studies of the methods proposed by the Referee for determination of esters in lemon extract be continued.

(5) That collaborative studies on the Seeker-Kirby Method for determination of esters in lemon and orange oils (Dept. Agr. Bull. **241**) be continued.

(6) That collaborative studies of extract mixtures containing both isopropyl alcohol and acetone be continued.

(7) That collaborative study of the photometric method for determination of vanillin and coumarin be continued.

(8) That work be continued on the determination of glycerol, vanillin, and coumarin in vanilla and imitation vanilla extracts with special reference to the automatic extraction of vanillin and coumarin.

(9) That the study of emulsion flavors be continued.

(10) That studies on maple concentrates and imitations be continued.

(11) That study of the method for determination of diacetyl, published in *This Journal*, **25**, 255, be continued.

FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That the procedures for sampling frozen pack fruit in barrels and in smaller containers, described in this year's report of the Associate Referee, be adopted as tentative and that further work on sampling of frozen pack fruit be discontinued.

(2) That an Associate Referee be appointed to initiate work in cooperation with the Referee, on methods of determining fruit and sugar content of frozen dessert fruits.

(3) That further collaborative study be made of the method for the electrometric titration of acidity.

(4) That further study be made of methods of separating fruit acids for subsequent titration.

(5) That studies of methods for the determination of sodium and chlorides be discontinued.

(6) That studies of polariscopic methods with particular reference to the possible interference of pectin be discontinued.

(7) That the methods developed by Hartmann for determination of citric, malic, and tartaric acids, published in *This Journal*, **26**, 444, be studied collaboratively.

(8) That the volumetric procedure for P_2O_5, **26.46, 26.47,** be adopted as official, final action.

(9) That the study of methods for the determination of water insoluble solids be continued.

SUGARS AND SUGAR PRODUCTS

It is recommended—

(1) That the study of unfermented reducing substances in molasses (*This Journal*, **29**, 242) be continued.

(2) That the study for the determination of moisture be continued.

(3) That tables of density of sucrose solutions at various temperatures be calculated.

(4) That the Zerban and Martin values for refractive indices of dextrose and invert sugar solutions (*This Journal*, **27**, 295) be adopted as tentative.

(5) That the study of the applicability of electro-deposition to the direct quantitative determination of dextrin in honey and honeydew honey (*Ind. Eng. Chem., Anal. Ed.*, **16**, 23–25) be continued.

(6) That a study of the characteristic properties of dextrins of honey and honeydew honey be undertaken with reference to their application to methods of analysis of honey.

(7) That the official method for the determination of free acid in honey, **34.99,** be studied collaboratively with a view to establishing the end point more accurately.

(8) That the method described in this year's report of the Associate Referee for the determination of resinous glaze in confectionery be subjected to collaborative study.

(9) That study be made on the determination of dextrose, maltose, and dextrins, by copper reduction methods in pure sugar mixtures.

(10) That the tentative methods, **34.133–34.155,** inclusive, be subjected to collaborative study.

(11) That the procedures in N.B.S. Circular C440, pp. 324–334, for measurement of transmission of solutions of commercial sugar products be considered with a view to their future adoption as tentative methods.

WATERS, BRINE, AND SALT

It is recommended—

(1) That methods for determination of fluorine in salt be studied collaboratively.

(2) That studies on boron be continued.

CHANGES IN OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE SIXTIETH ANNUAL MEETING, OCTOBER 14, 15, AND 16, 1946*

The changes in the methods of the Association recorded below become effective, as provided in section 8 of the by-laws, March 17, 1947, thirty days from the date of publication of this Report, Feb. 15, 1947.

There is appended a list of errata and emendations of the Sixth edition of *Methods of Analysis*.

1. SOILS

No additions, deletions, or other changes.

2. FERTILIZERS

(1) The following methods for recovery of platinum and preparation of platinum solution in the determination of potash were adopted as tentative.

I. RECOVERY OF PLATINUM

A. RECOVERY OF PLATINUM FROM THE ALCOHOL WASHINGS

(1) Allow the ammonium chloride washings to run into the flask with the alcohol washings. Allow the ammonium platinum chloride to settle, decant off the supernatant liquid, and save the platinum salt. Reduce as in B(1) or B(2).

(2) Evaporate the alcohol waste in a porcelain dish on a steam bath or an electric hot plate. (A piece of filter paper in the dish prevents most of the platinum from sticking to the dish.) Filter on a Büchner funnel and wash the reduced platinum. Transfer to a porcelain dish and ignite at about 700°C. in a muffle for about 20 minutes. Digest the reduced platinum in a porcelain dish on a steam bath with several portions of (1–3) hydrochloric acid. Repeat until the solution is colorless. Wash well with distilled water until a test with silver nitrate shows that all chlorides have been removed. Digest with a few portions of (1–4) nitric acid, wash, dry, and weigh.

(3) Acidify the alcohol waste with hydrochloric acid. Add either 20-mesh zinc, or aluminum in stick or sheet form (for volumes of 75 to 150 ml. of acid use 10 to 20 grams of metal) and allow to stand until all the platinum is reduced. Filter, ignite at 700°C. as in (2).

B. RECOVERY OF PLATINUM FROM THE K_2PtCl_6 SALT

(1) Dissolve the K_2PtCl_6 in 20 parts or more of hot water, acidify with hydrochloric acid and reduce with either 20-mesh zinc, or aluminum in sheet or stick form. Filter and ignite as in A 2.

(2) Dissolve the K_2PtCl_6 in water and precipitate as ammonium chloroplatinate with ammonium chloride. Allow to stand several hours, filter on a Büchner with suction, and wash with alcohol. Transfer to a porcelain dish and ash in a muffle, first at a low temperature (about 200°C.) for about 20 minutes and finally for 30 minutes at high heat (about 700°C.).

(3) Dissolve the K_2PtCl_6 in 20 parts or more of boiling water. Add sodium formate slowly, a pinch at a time, stirring well at each addition (excessive foaming may occur with a resultant loss of platinum unless great care is exercised). Complete reduction† is indicated by the solution becoming colorless. Filter and ignite as in A (2).

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* All references in this report are to *Methods of Analysis*, *A.O.A.C.*, 6th edition, 1945.

† Complete reduction may be tested as follows: Pipette about 25 ml of the clear solution into a 250 ml beaker, add a few drops of hydrochloric acid and small amount of potassium iodide solution. If platinum is not completely reduced, the solution will turn red unless a trace of nitric acid is present.

II. PREPARATION OF PLATINUM SOLUTION

Dissolve the platinum from I in a porcelain dish on a steam bath with three parts of conc. HCl and one part conc. HNO_3. After solution evaporate with additions of conc. HCl for three times, to remove the excess of HNO_3, and then with distilled water for three times, to remove the excess of HCl. Do not evaporate below $\frac{1}{4}$ the original volume. Filter and make to calculated volume. Evaporate and test a 10 cc portion, or one equivalent to one gram of platinum, for material insoluble in 80% alcohol. In case a blank shows the presence of impurities the platinum in the solution should be reduced, purified, and made up again. To determine the strength of the solution evaporate 2 ml in a porcelain dish with an excess of potassium sulphate of about 0.5 gm. Add alcohol and wash the K_2PtCl_6 as in the determination for potash. The solution may be made up so that 1 ml equals 1% K_2O in a 1 gm sample.

(2) The term "80% alcohol" in the method for potash sections **2.40** (p. 31) and **2.42** (p. 32) was editorially changed for classification, to "80% ethyl alcohol by volume."

3. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or other changes.

4. COSMETICS

(1) The following method for pyrogallol in hair dyes was tentatively adopted.

QUALITATIVE TEST

Add 5 to 10 ml of sample to separatory funnel containing ca 0.5 g of $NaHSO_3$ and extract with 2 or 3 successive 30-ml volumes of ether. Filter ether extracts thru cotton and evaporate to dryness on steam bath. Dry in oven at 100°C. for 30 to 60 min. Pulverize residue, mix well, and take melting point. If it does not melt between 131° and 134°C., sublime and again take melting point, which should fall within above range. Mix small portion of residue with equal quantity of sublimed pyrogallol and determine the melting point; it should not change.

QUANTITATIVE DETERMINATION

REAGENTS

Ferrous tartrate reagent.—Dissolve 1.00 g of sodium potassium tartrate (Rochelle salt) and 0.200 g of $FeSO_4 \cdot 7H_2O$ in water and dilute to 100 ml in a volumetric flask. PREPARE FRESH DAILY.

Sodium acetate soln.—Dissolve 15.0 g of $NaC_2H_3O_2 \cdot 3H_2O$ in water, bring to room temp. and dilute to 100 ml.

Preparation of standard curve.—To seven 100-ml volumetric flasks add from a buret 2.50, 5.00, 7.50, 10.00, 12.50, 15.00, and 17.50 ml of standard pyrogallol soln (Reagent grade, 0.2000 g/500 ml). Pipet into flasks 10 ml each of Na acetate soln and ferrous tartrate reagent, dilute to volume and mix thoroly. Fill one-half inch cell and read in neutral wedge photometer, or any suitable instrument, using filter centering at 560 millimicrons. Make photometer readings on each standard within 10 min. after color is developed. Draw standard curve on large scale graph paper so that the pyrogallol can be read to 0.01 mg. A straight line curve should be obtained between 1 and 7 mg. Below 1 mg the curve may slope away from straight line to zero point obtained by reading soln containing 10 ml each of ferrous tartrate reagent and Na acetate soln in 100 ml.

Extract a convenient aliquot of sample by one of following methods. (Usually 10 ml are sufficient.) In handling sample give it a minimum of exposure to air, as pyrogallol is readily oxidized.

(1) *Continuous extraction.*—Pipet sample aliquot into small continuous extractor containing ca 0.5 g of $NaHSO_3$. Extract with ether until pyrogallol is completely removed (3–7 hours, depending upon efficiency of extractor). Determine time required for each extractor under a certain set of conditions by extracting an aqueous soln of known pyrogallol content or by testing for complete extraction as follows: After the extraction is thought to be complete, remove flask containing ether and replace it with one containing fresh volume of ether and continue extraction for ca 30 min. Reserve main ether extract for treatment as directed in 3rd par. under "Extraction in Separatory Funnels," and treat second extract as follows: Evaporate ether on steam both to volume of ca 5 ml and continue evaporation without heat until odor of ether is gone. Dissolve residue in small volume of water and filter thru small paper into 50 ml volumetric flask. Wash extraction flask and paper thoroly with water. Dilute to volume and mix. Pipet 25 ml into 50-ml volumetric flask, add 5.00 ml each of Na acetate soln and ferrous tartrate reagent, make to volume and mix. Take photometer readings in one-half inch cell. The pyrogallol corresponding to this reading will equal total amount contained in extract. If color is too dark to read, repeat, using smaller aliquot. If pyrogallol obtained exceeds 0.5 mg (or a significant amount in comparison to that obtained in main extract) continue extraction with fresh volume of ether until less than that amount is obtained. Add total pyrogallol found to that determined in main ether extract.

(2) *Extraction in separatory funnels.*—Pipet sample into 125-ml separatory funnel containing ca 0.5 g of $NaHSO_3$, and extract 6 times with ether. For each extraction use a volume of ether equal to 3 or 4 times the volume of sample and shake vigorously for one min. Filter ether extracts thru cotton wet with ether. Make one or two additional extractions, filter thru same piece of cotton into separate beaker and use to test for complete extraction as directed under "Continuous extraction," beginning with "Evaporate ether. . . ."

Occasional samples that will emulsify so badly as to make extraction in continuous extractor impossible can be successfully extracted in separatory funnels by using volume of ether equal to 5 or 6 times the volume of sample.

Evaporate the main ether extract, from either method of extraction, on steam bath to volume of ca 5 ml and continue evaporation without heat until odor of ether is completely gone. (It is not necessary to evaporate to dryness.) Dissolve residue in water and wash completely into 100-ml volumetric flask. Dilute to volume and mix. If ether extract was colored green, indicating dissolved chlorophyll, add 2 ml alumina cream **34.19(b)** before diluting to volume. Filter thru dry paper and discard first 20 ml of filtrate. Pipet 5 or 10 ml into 100-ml volumetric flask and develop color as directed under "Preparation of Standard Curve," beginning, "Pipet into flasks. . . ." If photometer reading falls near lower end of scale, repeat, using larger aliquot; if color is too dark to read, dilute suitable aliquot (10–25 ml) to 100 ml in volumetric flask and use aliquot of diluted soln to develop color as directed above. From standard curve obtain amount of pyrogallol corresponding to photometer reading and calculate to gram/100 ml in original sample.

(2) The following method was adopted for resorcinol in liquid hair dyes (official, first action).

RESORCINOL IN HAIR LOTIONS

REAGENTS

(a) *Potassium iodide solution.*—Dissolve 25 g of KI in water and dilute to 100 ml.

(b) *Standard sodium thiosulfate solution.*—0.1 *N* $Na_2S_2O_3$, standardized by the method given in *Methods of Analysis, A.O.A.C.*, 6th Ed., **43.29.**

(c) *Standard bromide-bromate solution.*—0.1 bromine solution prepared and standardized as follows: Dissolve 3 g of potassium bromate and 15 g of potassium bromide in water and dilute to one liter. Pipet 25 ml of the soln into a 500-ml iodine flask and dilute with 100 ml of distilled water. Add 5 ml of hydrochloric acid, stopper the flask and shake it gently. Add 5 ml of potassium iodide soln and shake the mixture. Titrate with tenth-normal sodium thiosulfate soln using starch indicator.

DETERMINATION

Pipet 25 ml of sample into a 150-ml beaker. Dealcoholize as follows: Place the beaker on the covered surface of a steam bath and direct a current of air from a fan over the beaker. Evaporate to approximately 10 ml. Add 15 ml of water and again evaporate to 10 ml. Transfer the contents to a separatory funnel with several small portions of water to a final volume of 30 ml. Complete the transfer by washing the beaker with several portions of chloroform, totaling 25 ml. (Most of the solid material remaining after dealcoholization is transferred by the chloroform rather than the water.) Acidify with 1–2 ml of 10% hydrochloric acid.

Extract.—Extract with two additional 25 ml portions of chloroform. Wash each chloroform extract with the same 5 ml portion of water. Discard the chloroform. Add the 5 ml water wash to the residual acid aqueous layer. Extract the aqueous layer with five 35-ml portions of ethyl ether. Add 10 ml of water to the combined ether extracts. Evaporate the ether, at a low temperature, on the surface of a steam bath with the aid of a current of air from a fan. Transfer the water soln to a 100-ml volumetric flask, cool, make to volume, and mix.

Transfer a portion of this soln (the aliquot taken for assay should require from 20–40 ml of tenth-normal bromine) to an iodine flask. Add 50 ml of tenth-normal bromine soln. Dilute with 50 ml of distilled water. Add 5 ml of hydrochloric acid and immediately stopper the flask. Shake the flask and then allow it to stand for one minute. Remove the stopper just sufficiently to introduce 5 ml of potassium iodide soln taking care that no bromine vapors escape. Immediately stopper the flask. Shake thoroly, remove the stopper, and rinse it and the neck of the flask with 20 ml of distilled water. Titrate, *at once*, with tenth-normal sodium thiosulfate soln using starch indicator. Each ml of tenth-normal bromine is equivalent to 0.001835 g of resorcinol.

5. ENZYMES

No additions, deletions, or other changes.

6. INSECTICIDES AND FUNGICIDES

(1) The following directions for preparation of pure rotenone reagent were added to section **6.110** (p. 74).

REAGENT

From "C.P. Rotenone."—Dissolve rotenone in hot carbon tetrachloride, cool in refrigerator or ice-bath until precipitation of rotenone-carbon tetrachloride solvate has ceased. Filter thru Büchner funnel and wash once or twice with ice-cold carbon tetrachloride. Concentrate filtrate, crystallize, and filter as above described. Transfer crystalline residues to beaker, add ca twice their volume of alcohol, and heat nearly to boiling. It is not necessary that the crystals dissolve completely in alcohol. Cool to room temp., filter thru Büchner funnel, and draw air thru crystalline residue until most of alcohol is removed. Remove rotenone from funnel, dry in air, and

finally heat for 1 hour at 105°C. Mother liquors may be concentrated and rotenone-carbon tetrachloride solvate allowed to crystallize. The crystalline material may be used for further purification, or it may be kept for preparation of wash solns or for seeding to induce crystallization in the analytical procedure.

(2) The lead chlorofluoride method for fluorine, sections 6.18 and 6.19 (p. 58) was adopted as official, final action.

(3) The following directions for DDT were adopted as tentative.

DDT BY TOTAL CHLORINE

(1) Weigh a quantity of sample containing ca 1.00 g of DDT, transfer to 250-ml volumetric flask, and make to volume with chlorine and thiophene-free benzene. Shake until DDT is dissolved and soln well mixed. Transfer a 25-ml aliquot to 250–500 ml standard tapered Erlenmeyer flask.* Evaporate on steam bath until most of the benzene is removed. It is not desirable to evaporate to dryness, as DDT may decompose with loss of hydrochloric acid. Add 25 ml of 99% isopropanol and 2.5 g of metallic sodium in the form of ribbon or cut in small pieces, and shake flask to mix the sample with the alcohol. Connect to a reflux condenser and boil gently for at least ½ hour. Shake the flask occasionally. Eliminate excess metallic sodium by cautiously adding 10 ml of 50% isopropanol thru the condenser at the rate of 1–2 drops per second. Boil for an additional 10 min., and then add 60 ml of water, cool the flask, and transfer the contents to a 250-ml beaker. Add 2–3 drops of phenolphthalein soln, neutralize by adding nitric acid (1+1), and then add 10 ml in excess. Add a slight excess of N/10 silver nitrate and coagulate the precipitated silver chloride by digesting on a steam bath for ½ hour with frequent stirring. Cool, filter thru a fast qualitative paper, and wash thoroly with distilled water. Add 5 ml of saturated ferric alum soln, and determine the excess silver nitrate in the filtrate by titration with N/10 potassium thiocyanate. Subtract the quantity of silver nitrate found in the filtrate from that originally added. The difference will be that required to combine with the chlorine in the DDT. One ml of N/10 silver nitrate is equivalent to 0.0035457 g of chlorine. To obtain the percentage of DDT multiply the chlorine value by 2.

When electrometric titration is used proceed as directed under (1), thru "neutralize by adding nitric acid (1+1)," then add 6 ml excess. Cool the flask to room temp. and then transfer contents to a 400 ml beaker. The volume should be 200–250 ml. Titrate the Cl with N/10 silver nitrate using Ag-AgCl electrodes on an electrometric titrimeter (Fisher titrimeter or the equivalent). Calculate the percentage DDT as above.

NOTE: When the electrometric procedure is used the decolorizing carbon step in method (3) and the isoamyl alcohol-ethyl ether extraction methods (3), (4), and (5) may be omitted.

(4) The following methods were adopted tentatively for DDT in absence of other organic chlorine-containing compounds.

DDT IN ABSENCE OF OTHER ORGANIC CHLORINE-CONTAINING COMPOUNDS

Total Chlorine in Dusting Mixtures containing DDT in Absence of Organic Matter

(2) Weigh a quantity of sample containing about 0.75 g of DDT, transfer to a 100–200 ml volumetric flask, and add exactly 100 ml of chlorine and thiophene-free

* Weighing of the sample may be substituted for the aliquoting, provided the weighing does not introduce an error of more than 0.1% in the sample weighed.

benzene. Shake well until the DDT is dissolved and the soln is well mixed. Allow to settle and transfer a 10-ml aliquot to a 250–500 ml standard tapered Erlenmeyer flask. From this point proceed as directed in method (1) beginning "Evaporate on steam bath. . . ."

NOTE: If free sulfur is present use the hydrogen peroxide procedure outlined in method (6).

Total Chlorine in Dusting Mixtures Containing DDT in Presence of Organic Matter Such as Coloring Matter, Plant Resins, etc.

(3) Weigh a quantity of sample containing about 0.75 g of DDT and transfer to a 100–200 ml volumetric flask. Add a small amount of chlorine-free decolorizing carbon (0.5–1.0 g) and exactly 100 ml of chlorine- and thiophene-free benzene. Shake until the DDT is dissolved and the soln well mixed. Filter into a narrow-necked flask thru a fast qualitative paper without suction, keeping the funnel covered with a watch-glass to avoid loss from evaporation. Transfer a 10-ml aliquot to a 300-ml standard tapered Erlenmeyer flask. Proceed as in method (1) beginning with "Evaporate on steam bath . . ." thru "then add 60 ml of water . . . ." Cool, add 2–3 drops of phenolphthalein soln, neutralize by adding nitric acid (1+1) dropwise, and then add 10 ml in excess. Cool, if necessary, to room temp., transfer contents of flask and aqueous washings to a small separatory funnel, and shake with 15 ml of a mixture of equal volumes of isoamyl alcohol and ethyl ether. Draw off the aqueous layer into a second separatory funnel and extract again with 15 ml of the isoamyl alcohol-ethyl ether mixture. Draw off the aqueous layer into a 250-ml beaker. Wash the two extracts successively with 10 ml of water, and repeat with a second washing with another 10 ml of water. Combine the aqueous wash solns with the aqueous soln in the beaker. From this point proceed as directed in method (1) beginning "Add a slight excess of N/10 silver nitrate . . . ."

NOTE: Test the decolorizing carbon for the presence of chlorides by heating with dilute nitric acid (1+4), filtering, and adding silver nitrate soln to the filtrate. If chloride is present, wash with warm dilute nitric acid until washings no longer give a positive test.

Total Chlorine in Mineral Oil Sprays Containing DDT in the Absence of Organic Matter (Plant Extractive Material, Organic Thiocyanates)

(4) Transfer a quantity of sample containing 0.065–0.075 g of DDT to a 250–500 ml standard tapered Erlenmeyer flask. From this point proceed as directed under method (1) beginning "Add 25 ml of 99 per cent isopropanol . . . ."

NOTE: If DDT content is less than 2 per cent, use the isoamyl alcohol-ethyl ether extraction in method (3) to remove excess oil.

Total Chlorine in Mineral Oil Sprays Containing DDT and Organic Matter such as Plant Extractive Material from Pyrethrum or Derris and/or Cube

(5) Use method (4) with the isoamyl alcohol-ethyl ether extraction of method (3).

Total Chlorine in Mineral Oil Sprays Containing DDT in the Presence of Organic Thiocyanates With or Without Plant Extractive Material

(6) Transfer a quantity of sample containing 0.065–0.075 g of DDT to a 250–500 ml standard tapered Erlenmeyer flask. Add 25 ml of 99 per cent isopropanol and 2.5 g of metallic sodium in the form of ribbon or cut in small pieces, and shake flask to mix the sample with the alcohol. Connect to a reflux condenser and boil gently for at least ½ hour. Shake the flask occasionally. Eliminate excess metallic sodium by

cautiously adding 10 ml of 50% isopropanol thru the condenser at the rate of 1–2 drops per second. Boil for an additional 10 min. and then add 60 ml of water. Add 5 ml of 30-per cent hydrogen peroxide, a few drops at a time, thru the top of the condenser. Heat mixture in flask to boiling and boil for 15 min. Add 5 ml more of 30-per cent hydrogen peroxide and boil again for 15 min. Cool, add 2–3 drops of phenolphthalein soln, neutralize by adding nitric acid (1+1) dropwise, and then add 10 ml in excess. Cool, if necessary, to room temp., transfer contents of flask and aqueous washings to small separatory funnel, and shake with 15 ml of a mixture of equal volumes of isoamyl alcohol and ethyl ether. Draw off the aqueous layer into a second separatory funnel and extract again with 15 ml of the isoamyl alcohol-ethyl ether mixture. Draw off the aqueous layer into a 250-ml beaker. Wash the two extracts successively with 10 ml of water, and repeat with a second washing with another 10 ml of water. Combine the aqueous wash solns with the aqueous soln in the beaker. From this point proceed as directed in method (1) beginning "Add a slight excess of N/10 silver nitrate . . . " or electrometric titration as in method (1) may be run directly on this soln.

NOTE: Use the hydrogen peroxide and isoamyl alcohol-ether extraction procedure on dispersible powders or sprays that contain surface-active agents or other ingredients that react with silver nitrate.

A blank should be run on reagents used in these procedures. A.O.A.C. Methods **6.153** and **12.42**, or electrometric-titration methods may be substituted in the above procedures for the titration of chloride ion.

7. CAUSTIC POISONS

No additions, deletions, or other changes.

8. NAVAL STORES

No additions, deletions, or other changes.

9. GELATINE, DESSERT PREPARATIONS, AND MIXES

No additions, deletions, or other changes.

10. LEATHERS

No additions, deletions, or other changes.

11. TANNING MATERIALS

No additions, deletions, or other changes.

12. PLANTS

(1) The tentative methods for chlorophyll, sections **12.69–12.74**, inclusive (p. 140) were adopted as official, first action.

(2) The ortho-phenanthroline method for iron, sections **12.7–12.9**, inclusive, (p. 117) was made official, first action.

13. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

No additions, deletions, or other changes.

14. MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS

(1) The following tentative methods for malt were made official, first action:

Sampling, section **14.39** (p. 158).
Preparation of sample, section **14.40** (p. 159).
Bushel weight, section **14.41** (pl 159).
1,000 kernel weight, section **14.45** (p. 160).
Moisture in caramel malt and black malt, section **14.52,** (p. 161).
Diastatic power, section **14.61** (p. 166).

(2) The following official, first action, methods for malt were made official, final action: Moisture, sections **14.49–14.51,** inclusive (p. 161); color of wort, section **14.60** (p. 166).

(3) The following tentative methods for beer were adopted as official, first action:

Extract of original wort, section **14.7** (p. 151).
Real degree of fermentation or real attenuation, section **14.8** (p. 152).
Apparent degree of fermentation or apparent attenuation, section **14.9** (p. 152).
Total acidity-Indicator Titration Method, section **14.10** (p. 152).
Total acidity-Potentiometric Titration Method, section **14.11** (p. 152).
H-ion concentration (*p*H)-Electrometric Method, section **14.12** (p. 153).

(4) The following official, first action, methods for beer were adopted as official, final action:

Apparent extract or saccharimetric indication, section **14.4** (p. 151).
Iodine reaction for unconverted starch **(a)** for light beer, section **14.27** (p. 157).

(5) The following tentative methods for hops were adopted as official, first action:

Sampling, section **14.80** (p. 170).
Physical examination, section **14.81** (p. 170).
Preparation of sample for chemical analysis, section **14.82** (p. 171).
Moisture, section **14.83** (p. 172).
Resins, section **14.84–14.89,** inclusive (p. 172).

(6) The following tentative methods for brewing sugars and sirups were adopted as official, first action:

Extract, section **14.90** (p. 174).
Non-extract (apparent water), section **14.91** (p. 174).
Fermentable extract (b) rapid fermentation method, section **14.92** (p. 174).
Protein, section **14.93** (p. 175).
Iodine reaction for unconverted starch, section **14.95** (p. 175).
Acidity, section **14.96** (p. 175).
H-ion concentration (*p*H), section **14.97** (p. 176).
Ash, section **14.98** (p. 176).
Total reducing sugars, Munson-Walker method, section **14.99** (p. 176).

(7) The following tentative methods for wort were adopted as official, first action:

Specific gravity, section **14.103** (p. 176).
Original extract or original gravity, section **14.104** (p. 176).
Fermentable extract, section **14.105** (p. 176).
Iodine reaction, section **14.106** (p. 177).
Total acidity, section **14.107** (p. 177).
H-ion concentration (*p*H), section **14.108** (p. 177).
Color, section **14.109** (p. 177).
Protein, section **14.110** (p. 177).
Total reducing sugars, section **14.111** (p. 177).

(8) The following official, first action, methods for cereal adjuncts were adopted as official, final action:

Sampling, section **14.65** (p. 168).
Preparation of sample, section **14.66** (p. 168).
Physical characteristics, section **14.67** (p. 168).
Moisture, sections **14.68** and **14.69** (p. 169).
Oil or petroleum benzine extract, sections **14.70** and **14.71** (p. 169).
Extract, sections **14.72–14.75** (p. 169).
Crude fat or ether extract, section **14.76** (p. 170).
Protein, section **14.77** (p. 170).
Ash, section **14.78** (p. 170).
Crude fiber, section **14.79** (p. 170).

(9) The following procedures for the color determination of beer and wort were adopted tentatively:

Preparation of Dye Color Stock Soln:

The following amounts of the various dyes are dissolved in 200 ml of 95% ethyl alcohol and made up to one liter with distilled water. No mercuric chloride is added to this stock soln.

.4796 g. Amaranth WD (Red)
.9592 g. Tartrazine C Extra (Yellow) } per liter
.0840 g. Patent Blue VF (Blue)

A more concentrated Patent Blue is now also available. This suggests the preference of securing stock soln rather than experimenting with the mixture of the dyes. Since so little is required our supply will last for many years. We mention this since future batches of dyes may be still further varied. The above constitutes the stock soln which when diluted produces the Dye Color Reference Samples corresponding in color to what seems to be an average for American beers. If desired, the individual dyes may be dissolved separately, each in an aliquot third of the above quantities of alcohol in water. This permits slight variation in the combination of the dyes if, as in a few instances, slightly more of the red or other color seems to be required. Dye color solns prepared and standardized by Central Scientific Co., Chicago, Ill.

Preparation of Dye Color Reference Samples:

The above stock soln is prepared in such a manner that the number of ml diluted to 500 ml with water produces directly the desired Dye Color Reference Sample. Example: If 3 ml of stock soln is diluted to 500 ml with water, the 3° Dye Color Reference Sample is obtained. Two methods for performing this dilution are suggested.

Method A.—The individual reference samples can be prepared by pipetting out very carefully the exact amounts of dye corresponding to each of the color standards desired. For the range of 2° Lovibond to 5° in ¼° intervals, it would be necessary to use 2 ml, 2¼ ml, 2½ ml, etc., each diluted to 500 ml with water. To each 500 ml soln 10 ml of saturated mercuric chloride soln is added before final volume is completed. These standards can then be poured into the various special color bottles used in the procedure.

Method B.—The following simplified and possibly more easily controlled procedure for preparation of the Dye Color Reference Samples has been worked out; 12 ml of the stock soln is first diluted to one liter with distilled water, thus producing the 6° Dye Color Reference Sample. To prepare the usual set of Dye Color Reference Samples ranging from 2° to 5° in ¼° intervals as in the above, it is only necessary to measure into each bottle the following amounts of the 6° color sample:

Dye Color reference soln desired	2,	2¼,	2½,	2¾,	3,	3¼,	3½,	3¾,	4,	4¼,	4½,	4¾, 5
Volume of 6° color needed per 4 oz. bottle, ml	40,	45,	50,	55,	60,	65,	70,	75,	80,	85,	90,	95, 100

Into each bottle is also added 5 ml of saturated mercuric chloride soln and the bottle then filled to about ⅛ below the neck with distilled water. These bottles hold when completely full to the neck, from 122 to 123 ml. After a few trials it becomes easy to closely adjust to the 120 ml final dilution. Photoelectric values shown earlier in this study indicate no great discrepancy between sets of reference samples prepared in this way.

Further simplification of the dilution procedure can be worked out by pipetting equal quantities into several of the bottles and then removing portions from the bottles which are to become the lower values and adding them to the ones for the higher values.

Use of Dye Color Reference Samples:

Arrange the Dye Color Reference bottles from left to right beginning with the 2° color. In using these Reference Samples for beer color determination it is only necessary to slightly degas the beer so as to avoid excessive foaming (pouring from one beaker to another once). Undue degassing should be avoided to prevent turbidity formation. Comparison of the beer sample with the color reference samples can be done either by sliding the color bottles along in a rack on a table or bench exposed to northern light, or against a white blotter placed on the table in front of a fluorescent or other frosted white light. Comparison can also be made with the individual standard bottles. If the beer color appears to fall between two of the standards or closer to one, interpolation can readily be made. Thus, if the color is darker than 3° but not quite as dark as the 3¼°, a value of 3.15 might be assigned as the correct value.

Color of Wort Samples:

For wort color determinations it is necessary to filter the wort using a small amount of filter cel and a Reeve Angel 15 cm #202 filter paper. Sometimes a double filtration is required. The same set of colors and above procedure has been found to work out very well for plant wort. For laboratory wort, which often runs lower than the 2° color, it may be necessary to prepare additional Dye Color Reference Samples below the 2°, such as the 1¼°, 1½° and 1¾°. This can be done by diluting as indicated above. No loss in color appears to result by filtration of the wort.

Relation of Individual Reference Samples to Each Other:

For the purpose of comparing the color standards themselves as regards their keeping quality and to permit checking questionable beer colors, the following relationship has been found to exist when one reference color bottle is held sideways and compared with another held the long way:

Color as viewed thru—												
Thick Side	2,	2¼,	2½,	2¾,	3,	3¼,	3½,	3¾,	4,	4¼,	4½,	4¾,	5
Thin Side	1.20,	1.36,	1.53,	1.70,	1.88,	2.05,	2.23,	2.40,	2.57,	2.75,	2.93,	3.10,	3.27

Iodine Color Soln as Referee Checking Samples:

In the 1941 A.S.B.C. Proceedings, page 107, a table is given showing the relation between ml N/100 iodine per 100 ml water and the corresponding Lovibond values. This suggests a possible standard for use in checking the above Reference Samples. Although iodine does not produce colors exactly matching that of beer, it serves as a means of arriving at the degree of color. For closer matching we have found that the addition of copper sulphate and cobalt sulphate produces colors more closely resembling that of beer. Example: For a 4° Lovibond color standard use 4 ml of N/100 iodine per 100 ml water plus 10 ml of N/10 copper sulfate and 10 ml of cobalt sulfate. When copper and cobalt sulfates are present this raises the color value of the iodine 25%. The same simplified procedure can be used to prepare these iodine color mixtures as follows:

Prepare the 6° iodine color mixture by using 6 ml of N/10 iodine plus 150 ml N/10 $CuSO_4 \cdot 5H_2O$ plus 150 ml of N/10 $CoSO_4 \cdot 7H_2O$ made up to one liter with 2½% concentrated HCl. (It is found that such iodine color samples compare very closely with the corresponding Lovibond and Dye Color Reference Samples. These can only be used for immediate checking and comparison, as they do not keep well for more than a few days).

15. WINES

No additions, deletions, or other changes.

16. DISTILLED LIQUORS

The official, first action, methods for total acids **(16.10)** and fixed acids **(16.12)** were adopted as official, final action.

17. BAKING POWDERS AND BAKING CHEMICALS

(1) The gasometric methods for total carbon dioxide, sections **17.4–17.6,** inclusive (p. 208) and section **17.8** (p. 210) for residual carbon dioxide were adopted as tentative for baking powders containing added calcium carbonate with the provision of optional use of HCl (1+2) or H_2SO_4 (1+5).

(2) The method for available carbon dioxide, section **17.9** (p. 210) was adopted as tentative for baking powders containing added calcium carbonate.

18. COFFEE AND TEA

(1) The phrase "not applicable to coffee extracts" was inserted below the title of the Power-Chesnut method, section **18.14** (p. 217), first action.

(2) The Fendler-Stüber method (modified) for caffeine in coffee, section **18.15** (p. 217) was made official, first action.

(3) The Bailey-Andrew method for caffeine in tea, section **18.41** (p. 220) was made official, first action, for caffeine in coffee.

19. CACAO BEAN AND ITS PRODUCTS

(1) The words "ca 20° (below 25°) in line 7, paragraph 3 of section **19.16** (p. 227) were changed to read "20° or lower."

20. CEREAL FOODS

(1) The word "wheat" was added to the title on page 259, heading sections **20.70–20.76,** inclusive.

(2) The official, first action method for original ash of flour in phosphated and self-rising flour, section **20.8** (p. 238) was made official, final action.

(3) The official, first action, dry ashing method for iron, sections **20.9–20.12,** inclusive (p. 238), was made official, final action, and was adopted as official, first action, for macaroni products, degerminated and whole corn meals, applicable to enriched products.

(4) The following wet ashing method for iron was adopted as official, first action, for flour and bread, applicable to enriched products:

WET DIGESTION METHOD FOR FE

REAGENTS

HNO_3 and H_2SO_4, Fe free or nearly so.

2 M NaAc. $3H_2O$ buffer soln.—(272g/1).

0.1% orthophenantholine.—See dry ashing method for enriched flour.

10% hydroxylamine hydrochloride.—See dry ashing method for enriched flour.

PROCEDURE

Transfer 10.00 g of flour or air-dried ground bread into a 800 ml Kjeldahl flask, previously rinsed with dilute acid, followed with distilled H_2O, add 20 ml H_2O, mix, pipet 5 ml H_2SO_4 into flask, mix, add 25 ml HNO_3, mix well. After standing a few minutes, heat very gently at brief intervals to avoid foaming out of the flask until after the heavy evolution of NO_2 fumes ceases, continue to heat gently until the material begins to char, add cautiously a few ml of HNO_3 at intervals until SO_3 fumes evolve and a colorless or very pale yellow liquid is obtained (60–65 ml HNO_3 in all. (Time about 2 hours). Cool, add 50 ml H_2O, one Pyrex glass bead, heat to SO_3 fumes, cool, add 25 ml H_2O, and filter thru 11 cm filter into 100 ml volumetric flask, rinse out the flask, cool, and make to mark. Pipet 10 ml in 25 ml volumetric flask, add 1 ml hydroxylamine, rotate and stand a few minutes, add 9.5 ml 2 *M* NaAc soln, and 1 ml o-phenanthroline, make to mark and mix, stand at least 5 minutes or longer, and read in 2" cell on neutral wedge photometer or some other instrument of similar precision. (On self-rising flour the 9.5 ml of 2 *M* NaAc may be reduced to 8.0 ml.) The exact amount of 2 *M* sodium acetate buffer needed for each digest to adjust to the most deisrable *p*H range may be determined on 10 ml aliquot of the sample with the 2*M* buffer soln and diluted with water to 25 ml. Determine *p*H electrometrically or by comparison with an equal volume of a buffer soln of *p*H 3.5 (6.4 ml of 2 *M* NaAc · 3 H_2O (272 g/1) and 93.6 ml of 2 *M* acetic acid (120 g/1) and diluted to one l and five drops of bromophenol blue. Although color development will occur from *p*H 2 to *p*H 9 it is desired to avoid a *p*H lower than 3.0 and preferably

to work between 3.5 and 4.5. If the details are followed on cereal products, the 9.5 ml of 2 *M* NaAc buffer soln is satisfactory. On samples high in iron an aliquot of 5 ml instead of 10 ml can be used with 4.8 ml of the 2 *M* buffer soln. Digestion must be carried out to avoid any contamination with iron. A blank should be run. After correction for blank calculate as mg/lb of Fe.

(5) The official, first action, method for calcium, section **20.13** (p. 240), was adopted as official, final action.

(6) The official, first action, electrometric method for H-ion concentration, section **20.27** (p. 244), was adopted as official, final action.

(7) The official, first action, methods for moisture, section **20.106** (p. 263), and for fat, section **20.107,** in fig bars and raisin-filled crackers, were adopted as official, final action.

(8) The tentative method for total solids in raisin bread, section **20.84(b)** (p. 261 was adopted as official, first action, after substitution of an uncovered for the covered dish for the drying of the air-dried sample in the vacuum oven for about 16 hours at 70° under a pressure not to exceed 50 mm. of Hg.

21. COLORING MATTERS

No additions, deletions, or other changes.

22. DAIRY PRODUCTS

No additions, deletions, or other changes.

23. EGGS AND EGG PRODUCTS

No additions, deletions, or other changes.

24. FISH AND OTHER MARINE PRODUCTS

No additions, deletions, or other changes.

25. FLAVORING EXTRACTS

No additions, deletions, or other changes.

26. FRUITS AND FRUIT PRODUCTS

(1) The official, first action, volumetric method for phosphoric acid, section **26.46, 26.47** (p. 398), was adopted as official, final action.

(2) The following procedures for sampling frozen pack fruit in barrels and smaller containers were adopted as tentative:

SAMPLING

(1) *Barrels.*—Use a stainless steel or corrosion-resistant tube approximately $1\frac{1}{4}''$ in diameter and 36″ long, one end of which is serrated and set to run freely, the other end of which has a removable cap and arrangement for use of electrical energy in drilling. To assist in removal of core samples obtain a wooden ram smaller in diameter but longer than the tube.

Remove the bottom of the barrel and take three cores evenly spaced around its

circumference near the chime parallel to the length of the barrel and thru its entire length. Take a fourth core at the approximate center of the barrel.

(2) *Small containers (approx. 30 to 50 lbs.).*—Use a modified corrosion-resistant auger 1 to 1½″ in diameter and 19″ long which can be operated by an electric motor. The auger should have no lead screw or cutters and the angle of the face should not be flat but approximately 170–175°. A corrosion-resistant sampling can open at one end approximately 6″ diameter, 4″ high, with an outlet at the other end about 1″ long of a diameter slightly larger than that of the auger set near the circumference is used with the auger for collection of the borings.

Place sampling can on surface of frozen fruit operating auger thru the small opening at the bottom. Take three vertical cores evenly spaced about the circumference and approximately ½″ from the edge of the container and take one core at or near the center. Use sampling can in conjunction with the auger and remove both simultaneously to prevent borings from falling through delivery outlet.

27. GRAIN AND STOCK FEEDS

The following official, first action, methods were adopted as official, final action:

Sampling, section **27.1** (p. 404).
Water-soluble acidity, section **27.41** (p. 414).
Rice hulls in rice bran, section **27.43** (p. 414).
Oat hulls in oats and oat feeds, section **27.44** (p. 414).

28. MEAT AND MEAT PRODUCTS

No additions, deletions, or other changes.

29. METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

No additions, deletions, or other changes.

30. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

31. OILS, FATS, AND WAXES

(1) The tentative S.P.A. method for unsaponifiable residue, section **31.40** (p. 504) was adopted as official, first action.

(2) The official F.A.C. method for unsaponifiable residue, section **31.37–31.39**, inclusive (p. 504), was deleted, first action.

(3) The tentative method for squalene, section **31.41–31.43,** inclusive (p. 505), was adopted as official, first action.

32. PRESERVATIVES AND ARTIFICIAL SWEETENERS

The statement "Quantitative-see 29.22–29.33, inclusive" was inserted following section **32.30** (p. 533) for cross reference.

33. SPICES AND OTHER CONDIMENTS

(1) The methods for free mineral acids in vinegar, sections **33.85–33.87,** inclusive (p. 554), were deleted.

(2) The following method for starch in salad dressings was adopted as tentative:

STARCH IN SALAD DRESSING

REAGENTS

(1) *Calcium chloride soln.*—30 g per 100 ml of soln, adjusted to approximately 0.01 *N* alkalinity.

(2) *Alcohol.*—95%.

(3) *Alcohol.*—70%.

(4) *Alcohol-sodium hydroxide soln.*—70 ml of 95% alcohol plus 25 ml of 0.1 *N* NaOH.

(5) *Ammonium sulfate soln.*—Saturated aqueous soln.

(6) *Iodine potassium iodide soln.*—2 g of I plus 6 g of KI in 100 ml of water.

PROCEDURE

Determine the total acidity of the prepared sample as directed in *Methods of Analysis, A.O.A.C.*, 1945, **33.51.** Place 4–5 g of the prepared sample in a 500-ml Erlenmeyer flask, add the calculated quantity of 0.1 *N* NaOH to neutralize the acid in the weight of sample taken; and add 100 ml of the $CaCl_2$ soln. Stopper the flask and swirl gently until all large lumps of mayonnaise (or salad dressing) are broken. Add glass beads. Wet the inside of a reflux condenser and the stopper with water, allow to drain for 1 min., then connect flask to condenser. Place an asbestos board with a hole in the center under the flask and boil gently for 30 min., swirling from time to time. (Slight frothing occurs in the first few minutes.) Leaving the condenser connected, cool the flask and contents to room temp. in a pan of ice-water. Remove the flask, stopper it, and shake vigorously. Pour the contents into a centrifuge bottle[1] and centrifuge for about 10 min. at 1,500 r.p.m. Pipet a 50-ml aliquot into a centrifuge bottle containing 150 ml of 95% alcohol, stopper (rubber)[2] and shake vigorously for ca 5 min. Rinse particles adhering to stopper into bottle with 70% alcohol; use same rubber stopper thruout the following steps. Centrifuge at 1,500 r.p.m. for ca 10 min. Decant the supernatant liquid thru an asbestos pad in a Caldwell crucible, using suction; retain the precipitated starch in the centrifuge bottle, adding 70% alcohol and recentrifuging if necessary. Pour off the supernatant liquid as completely as possible without transferring the starch to the crucible; 70% alcohol may be used in rinsing the mouth of the centrifuge bottle, and the washings may be allowed to pass thru the Caldwell crucible. Remove the asbestos pad and transfer it to the centrifuge bottle. Rinse particles adhering to the crucible into the bottle with water. Add water to about 100 ml, stopper, shake bottle vigorously to break up precipitate, then add an excess[3] of the I-KI soln (2–3 ml) and 50 ml of the $(NH_4)_2SO_4$ soln. Stopper, and shake bottle vigorously. Rinse particles adhering to stopper into centrifuge bottle with water, and centrifuge until clear. Decant supernatant liquid with suction thru an asbestos pad in a Caldwell crucible. Add 50 ml of the alcohol-NaOH soln to the precipitate in the centrifuge bottle, stopper, and shake vigorously; wash stopper with 70% alcohol, centrifuge, and decant thru the same pad as before. Repeat treatment with the alcohol-NaOH soln until practically all the blue color disappears (usually 2–3 treatments are sufficient). Without centrifuging, transfer contents of the centrifuge bottle to the Caldwell crudible, using 70% alcohol to rinse the centrifuge bottle. Aspirate until pad is dry, then transfer pad to a 500-ml Kjeldahl flask. Add 10 ml of HCl (sp. gr. 1.1029) to the centrifuge bottle, stopper, and shake vigorously until all starch particles adhering to walls are washed free or are in soln. Transfer the HCl to Kjeldahl flask. Rinse cen-

[1] Wide-mouthed 250-ml bottles are the most convenient type. By tilting the bottle after centrifuging a 50-ml aliquot may be removed without including any of the oil-containing upper layer.

[2] A tight-fitting stopper that extends only 3–4 mm into the mouth of the centrifuge bottle is preferable.

[3] Excess present when supernatant liquid after centrifuging has straw to brown color. If supernatant liquid is colorless, more of the I-KI solution must be added.

trifuge bottle and crucible with small portions of water, adding washings to contents of Kjeldahl flask until the total volume reaches approximately 60 ml. Loosen particles adhering to the inside of the Caldwell crucible with the aid of a rubber policeman. Attach Kjeldahl flask to a reflux condenser, first adding glass beads to lessen bumping, place an asbestos board with a hole in the center under the flask, and boil for 1 hour. Cool, nearly neutralize with 1+1 NaOH (use methyl orange as indicator), and filter into a 200-ml volumetric flask. Wash Kjeldahl and contents of filter paper with water until volume reaches 200 ml. Mix well and determine dextrose as directed in **34.38.** Dextrose $\times 0.9$ = starch. Correct for added volume of 0.1 *N* NaOH used to neutralize acidity of salad dressing. Report percentage of starch in sample.

(3) Section **33.42** (p. 546) of the tentative method for starch was revised as follows and titled to show its applicability to both prepared and dry mustards.

Place 5 g of prepared mustard,* or 2–3 g of dry mustard flour into 500-ml Erlenmeyer flask and add 100 ml of the calcium chloride soln from a pipet, swirling the flask gently until all lumps are broken. Add the calculated quantity of N/1 NaOH to neutralize acid in weight of prepared mustard taken for analysis. Add glass beads. Connect to reflux condenser, first wetting inside of condenser and stopper with water and draining one min. Heat gently (on asbestos board with hole in center) to avoid initial foaming, and boil for 15 min. Leaving condenser connected, cool flask to room temp. in a pan of cold water. Remove flask, stopper, and shake vigorously. Pour contents into centrifuge bottle and whirl at 1,500 r.p.m. for 5 min. Withdraw as much as possible of the partially clarified middle layer (ca 75 ml). Filter thru a small pledget of cotton placed in a funnel. Pipet 50 ml of filtrate into the second centrifuge bottle containing 150 ml of 95% alcohol. Stopper and shake vigorously for several minutes. Centrifuge at 1,500 r.p.m. until clear (ca 5 minutes). Decant liquid thru an asbestos pad in a Caldwell crucible, using suction, without transferring starch to crucible. Transfer pad to same centrifuge bottle, and rinse all particles adhering to crucible into bottle with water. Add water to volume of ca 100 ml. Stopper and shake bottle vigorously, until precipitate is finely dispersed. Add a slight excess of I-KI soln (2–3 ml) and 30 ml of saturated ammonium sulfate soln. Stopper and shake bottle. Rinse particles adhering to stopper into bottle and centrifuge until clear. Decant supernatant liquid, with suction, thru asbestos pad in a Caldwell crucible. Add 50 ml of alcohol-NaOH soln to precipitate in centrifuge bottle. Stopper and shake vigorously. Wash stopper with 70% alcohol. Centrifuge and decant supernatant liquor thru same pad as before. Repeat treatment with alcohol-NaOH soln until practically all of blue color disappears (usually 2–3 treatments are sufficient). Without centrifuging, transfer contents of bottle to Caldwell crucible, using 70% alcohol. Aspirate until pad is dry, then transfer pad to a 500-ml Kjeldahl flask. Rinse bottle and crucible with 10 ml of HCl (sp. g. 1.1029) followed by five 10-ml portions of water, carefully removing all adhering particles. Attach Kjeldahl flask to reflux condenser, first adding glass beads to lessen bumping. Place on an asbestos board with hole in center and boil for one hour. Cool, neutralize with 1+1 NaOH (methyl orange) and filter into 200-ml volumetric flask; rinse flask and filter thoroly, and make to volume with water. Mix well, and determine dextrose on 50-ml aliquot by **34.39.** (Blank or Fehling soln should not exceed 0.3 mg.)

$$\% \text{ starch} = \frac{\text{g dextrose} \times 0.9(100+A+B) \times 0.8}{\text{weight sample}}$$

* With prepared mustard the acidity, **33.39**, and solids, **33.35**, values are necessary in the procedure and calculations.

wherein A = ml N/1 NaOH used to neutralize acidity of prepared mustard, and B = H_2O in sample taken.

(4) The official method for copper-reducing substances by direct inversion, section **33.40** (p. 545) was deleted, first action.

34. SUGAR AND SUGAR PRODUCTS

The accompanying table of Zerban and Martin of refractive indices of dextrose and invert sugar was tentatively adopted.

35. PROCESSED VEGETABLE PRODUCTS

(1) The following method for catalase in frozen vegetables was adopted as tentative:

CATALASE

Titrimetric Method[a]

REAGENTS

(1) Relatively stable reagents—

(a) *Calcium carbonate.*—Use reagent grade.

(b) *0.1 M phosphate buffer, pH 7.0.*—Dissolve 1/15 mol. of dibasic phosphate and 1/30 mol. of monobasic phosphate in water and dilute to 1 liter (*e.g.*, 15.22 g of $K_2HPO_4 \cdot 3H_2O$ and 4.54 g of KH_2PO_4 per liter) or mix 0.1 *M* soln of the two phosphates in the ratio two volumes of dibasic to one volume of monobasic phosphate. The *p*H should be between 6.8 and 7.1.

(c) *2 N sulfuric acid containing molybdate.*—Add 55 ml of concentrated sulfuric acid to about 800 ml of water, cool, add 0.1 g of finely ground $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, agitate until completely dissolved and dilute to 1 liter with water.

(d) *Sodium thiosulfate (ca 0.01 N) in 10% potassium iodide.*—Dissolve 100 g of KI, 2.50 g of $Na_2S_2O_3 \cdot 5H_2O$, and about 1 g of Na_2CO_3 in 500 ml of water and dilute to 1 liter. This soln need not be standardized.

(e) *Iodine soln (0.01 N).*—Dissolve 1.27 g of I and 2 g of KI in 10 ml of water and dilute to 1 liter. Standardize against standard $Na_2S_2O_3$ soln.

(2) Relatively unstable reagents—

(a) *0.1 N hydrogen peroxide.*—Dilute 0.58 ml of 30% H_2O_2[b] to 100 ml. with cold water. Keep in refrigerator or ice bath while not in use. PREPARE FRESH DAILY.

(b) *2% dextrose in buffer.*—Dissolve 20 g of dextrose in 100 ml of the 0.1 *M* phosphate buffer described in 1 (b) above. Keep refrigerated. Prepare fresh weekly.

(c) *Starch soln.*—Add 1.0 g of soluble starch to 100 ml of cold water, stir thoroly, and heat to boiling. Prepare fresh biweekly.

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[a] The procedure described in (a) differs from that described in (b) only to the extent that it may be carried out at any temperature between 0° and 20° C. Catalase activity is about twice as great at 20° as at 0° but nonenzymic decomposition of H_2O_2 is also greater, so that the reliability of the method for detection purposes is about the same over this temperature range. Higher temperatures are unsatisfactory not only because of nonenzymic H_2O_2 decomposition but also because the destruction of catalase by H_2O_2, which is somewhat evident at 20°, becomes marked.

[b] It is occasionally stated that hydrogen peroxide with stabilizer should not be used in the catalase test. The authors are not aware of any data supporting this statement though they prefer to use 30% hydrogen peroxide, which contains no preservative. Limited tests concerning the effect of hydrogen peroxide stabilizers on spinach catalase have revealed that the activity is unaffected by either acetanilide (the most common stabilizer) or acetamide in 100 times the concentration that would be present in the test mixture if it were made with 3% hydrogen peroxide containing the U.S.P. tolerance (50 mg per 100 ml) of stabilizer. This finding should not be considered a recommendation that stabilizer-containing hydrogen peroxide be used, since the study has not included the behavior of catalases from other sources (*e.g.*, animals) or the effect of impurities in the stabilizers added to the hydrogen peroxide.

Refractive Indices of Dextrose and of Invert Sugar Solutions at 20°C[1]

PER CENT SUGAR BY WEIGHT IN AIR	REFRACTIVE INDEX		PER CENT SUGAR BY WEIGHT IN AIR	REFRACTIVE INDEX	
	DEXTROSE	INVERT SUGAR		DEXTROSE	INVERT SUGAR
0	1.33299	1.33299	40	1.39872	1.39866
1	.33442	.33441	41	.40061	.40056
2	.33587	.33583	42	.40252	.40248
3	.33732	.33727	43	.40444	.40440
4	.33878	.33872	44	.40637	.40634
5	.34025	.34018	45	.40832	.40830
6	.34173	.34165	46	.41028	.41026
7	.34322	.34313	47	.41225	.41225
8	.34472	.34462	48	.41424	.41424
9	.34623	.34612	49	.41624	.41625
10	1.34775	1.34764	50	1.41826	1.41827
11	.34929	.34916	51	.42029	.42031
12	.35083	.35070	52	.42233	.42236
13	.35238	.35225	53	.42439	.42443
14	.35394	.35381	54	.42646	.42650
15	.35552	.35538	55	.42854	.42860
16	.35711	.35696	56	.43064	.43070
17	.35870	.35856	57	.43276	.43283
18	.36031	.36016	58	.43488	.43496
19	.36193	.36178	59	.43703	.43711
20	1.36356	1.36341	60	1.43918	1.43928
21	.36520	.36506	61	.44136	.44146
22	.36686	.36671	62	.44354	.44365
23	.36852	.36838	63	.44574	.44586
24	.37020	.37006	64	.44796	.44808
25	.37189	.37175	65	.45019	.45032
26	.37359	.37345	66	.45243	.45257
27	.37530	.37517	67	.45469	.45484
28	.37703	.37690	68	.45697	.45712
29	.37877	.37864	69	.45926	.45941
30	1.38052	1.38040	70	1.46156	1.46172
31	.38228	.38217	71	.46388	.46405
32	.38406	.38395	72	.46621	.46639
33	.38584	.38574	73	.46856	.46874
34	.38765	.38755	74	.47092	.47111
35	.38946	.38937	75	.47330	.47350
36	.39129	.39120	76	.47570	.47590
37	.39313	.39305	77	.47810	.47831
			78	.48053	.48074
38	.39498	.39491	79	.48297	.48319
39	.39684	.39678	80	1.48542	1.48564

[1] Zerban and Martin, J. Assoc. Official Agr. Chem. *27*, 295 (1944).

PREPARATION OF EXTRACT OF VEGETABLES

Comminute 50-g portions of undried vegetable in a Waring blendor for three min. with about 1 g of calcium carbonate and sufficient water to make a total volume of 200 ml. Remove the larger solid particles by filtration thru a gauze-backed cotton milk filter. Assay the filtrate for catalase activity within 30 min. For dried vegetables 5 g of sample should be extracted. (Rehydrated before extraction.) Samples should be handled *only* with non-metallic or stainless steel spatules in order to avoid contamination with oxides of heavy metals.

DETERMINATION

(a) *To demonstrate the presence or absence of catalase* (*Note* [a], p. 76).—To 10 ml (or less, depending on activity) of the extract add water to give a volume of 43 ml and 5 ml of buffered dextrose soln. Mix and add 2 ml of 0.1 *N* H_2O_2. *Immediately* after addition of the H_2O_2 thoroly mix and quickly remove a "zero-time" aliquot of the completed reaction mixture with a rapid-flow pipet, and blow it into a 125-ml Erlenmeyer flask that contains 10 ml of the H_2SO_4-molybdate soln. Zero time is the time that delivery of the aliquot from the pipet is started. Remove 10-ml aliquots at 5 and 10 min. The temperature of the reaction mixture must remain at less than 20°C. At any time within an hour add to each flask 5 ml of the $Na_2S_2O_3$-KI soln and mix. After standing for 3 to 5 min., titrate the excess $Na_2S_2O_3$ with 0.01 *N* iodine[a], using about 10 drops of 1% starch as indicator.

A blank is run in exactly the same manner as just described, except that no H_2O_2 is added (water is added instead) and the 5- and 10-min. aliquots are not run. The differences between the titer value of the blank and of the values obtained in presence of H_2O_2 are the iodine-soln equivalents of the H_2O_2 present at the respective times. The "zero-time" titer value should be between 0.5 ml and 2 ml of iodine soln; the differences between the blank-titer value and the "zero-time" titer value should be 3.0–4.5 ml of 0.01 *N* iodine. A difference of less than 3.0 ml (*i.e.*, iodine titer values greater than 2 ml) indicates that the H_2O_2 was too weak or that the catalase activity was so high that a large amount of H_2O_2 was decomposed before the "zero-time" aliquot was removed. In the latter case the differences corresponding to 5 and 10 min. may be nearly 0, although large amounts of catalase are present. In such a case the catalase content of the extract can be determined by procedure (b) if desired. Where 10-ml aliquots of extract are used, and when the blank-titer value is 4–6 ml and the "zero-time" titer value is <2 ml, the catalase is indicated to be absent within experimental error when titer values for 5 min. and 10 min. do not differ from the zero-time titer value by more than 0.20 ml and 0.40 ml, respectively.

(b) *To accurately determine the catalase content of a sample in terms of* K_f.—The "Katalase Fähigkeit," K_f, is k, the first order reaction constant log base 10 determined at 0°C., divided by the grams of sample per 50 ml of reaction mixture. That is, $K_f = k$ per g and is therefore an expression of the catalase content or purity of the preparation. Pure catalase has a K_f of 40,000 to 60,000, depending on the source. If K_f is to be determined, the assay is made exactly as described in (a) except that the reaction mixture is maintained at 0°C. It is generally desirable to obtain a 15-min. titer value in addition to those described in (a). If the sample is an enzyme preparation of high activity rather than a vegetable, a suitable amount of it should be dissolved in dilute buffer, *p*H 6.5–7.5, preferably containing 2% dextrose.

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[a] Due caution should be taken to avoid exposure of the dilute iodine solution to excessive light even while in the burette.

CALCULATION OF K

The value of K_f for the 0 to 5-min. period and the 5- to 10-min. period should check at 0°C., but at higher temperatures K_f (5′ to 10′) may be lower than K_f (0′ to 5′), as catalase is inactivated by H_2O_2 at a significant rate at the higher temperatures. K_f is given by the following formula:

$$K_f = \frac{\left(\frac{1}{t_b - t_a}\right) \log \left(\frac{\text{titer No } H_2O_2 - \text{titer at } t_a}{\text{titer No } H_2O_2 - \text{titer at } t_b}\right)}{\text{gms of sample per 50 ml reaction mixture}}$$

where t_a and t_b are the initial and final times for the two titer values under consideration. For extracts of undried vegetables prepared as described earlier, the grams per reaction mixture are obtained with sufficient accuracy by multiplying the ml of extract used by 0.25. For most accurate results the enzyme concentration should be adjusted so that the difference between the blank titration (No H_2O_2) and the t_0′ titration is 3 to 4 ml, and between the blank titration and the t_{10}′ titration is 0.5 ml to 1 ml of iodine solution.

36. VITAMINS

(1) The tentative methods for riboflavin, sections **36.32–36.40,** inclusive, (pp. 613–617) were deleted.

(2) The following method for riboflavin was tentatively adopted:

RIBOFLAVIN

PREPARATION OF SAMPLE SOLUTION

Conduct the following operations at all stages of the process so that the solns are protected as far as possible from light which destroys riboflavin.

Grind the sample to pass thru a sieve having circular openings 1 mm in diameter and mix thoroly. If the sample cannot be ground, reduce it to as fine a condition as possible. (For tablet or capsule vitamin preparations reduction of the sample to as fine a condition as possible may be necessary when disintegration does not occur upon autoclaving.)

The quantity of material taken for assay depends upon the amount available and the expected riboflavin content. In general, weigh an amount of sample that contains about 5 to 50 micrograms of riboflavin, transfer to a suitable size flask and suspend in a volume of 0.1 *N* HCl equal in ml to not less than 10 times the dry weight of sample in grams. Autoclave at 15 pounds pressure (121.5°C.) for 30 minutes and cool to room temp. Adjust the soln to *p*H 5–6 with NaOH soln, then add diluted HCl soln to the point of maximum precipitation of the dissolved protein (at the isoelectric point of the protein, about *p*H 4.5). Dilute the suspension to a measured volume that contains more than 0.1 microgram of riboflavin per ml. Filter thru paper known not to adsorb riboflavin, and test for the presence of dissolved protein in the clear filtrate, at a *p*H both below and above that used, by adding dropwise first diluted HCl, then diluted NaOH. If further precipitation occurs the soln must again be adjusted to the point of maximum precipitation, then filtered. To a measured volume of the clear filtrate add diluted NaOH soln to a *p*H of 6.6 to 6.8, again filter if cloudiness occurs, then dilute to a final volume that is expected to contain 0.1 microgram of riboflavin per ml.

For samples of riboflavin content too low to meet these requirements, the following modification is necessary. The clear filtrate, after removal of precipitated protein at *p*H 4.5, is concentrated to a suitable volume with heat under reduced pressure and filtered if necessary.

Standard riboflavin soln.—Dissolve 50 mg of U.S.P. Riboflavin Reference Standard, accurately weighed, in sufficient dilute acetic acid (1.2 ml of glacial acetic acid to 1,000 ml of distilled water) to make 500 ml. Preserve this stock soln, protected from light, and under toluene, in a refrigerator. Prepare the Standard Soln by diluting 1 ml of the stock soln with sufficient distilled water to make 1000 ml, representing 0.1 microgram of U.S.P. Riboflavin Reference Standard in each ml. Prepare fresh Standard Soln for each assay.

Basal medium stock soln—	
Photolyzed peptone soln	50 ml
Cystine soln	50 ml
Yeast supplement soln	5 ml
Dextrose anhydrous	15 gm
Salt Soln A	2.5 ml
Salt Soln B	2.5 ml

Dissolve the anhydrous dextrose in the solns previously mixed and, if necessary, adjust to a *p*H of 6.8 using dilute sodium hydroxide soln. Finally, add sufficient distilled water to make 250 ml of soln.

Photolyzed peptone soln.—Dissolve 40 gm of peptone in 250 ml of distilled water, and 20 gm of sodium hydroxide in 250 ml of distilled water, mix the solns in a crystallizing dish having a diam. of 25 cm. At a distance of about 1 foot from the dish place a 100-watt bulb fitted with a reflector, and expose the soln to light from the bulb for 6–10 hours, then allow the mixture to stand for the remainder of 24-hour period. Maintain the soln during this treatment at a temp. not exceeding 25°C. Neutralize the sodium hydroxide with glacial acetic acid and add 7 gm of anhydrous sodium acetate and sufficient distilled water to make the soln measure 800 ml. Preserve the soln under toluene in a refrigerator.

Cystine soln.—Dissolve 1 gm of l-cystine in 20 ml of 10% hydrochloric acid and add sufficient distilled water to make the soln measure 1000 ml. Store the soln under toluene in a refrigeragor not below 10°C.

Yeast extract soln.—Heat a mixture of 500 gm of fresh baker's yeast (starch-free) and 5 liters of distilled water in flowing steam for 2 hours, then autoclave it at 15 pounds pressure (121.5°C.) for 40 min. Allow the mixture to settle, filter, and evaporate the filtrate to a volume of 125 ml under reduced pressure, at a temperature not exceeding 50°C.

Yeast supplement soln.—Add 125 ml of an aqueous soln containing 38 gm. of lead subacetate to 125 ml of the yeast extract soln. Filter, and add dilute ammonia (containing not less than 9.5% and not more than 10.5% NH_3) to the filtrate to produce a *p*H of approximately 10. Filter, and add glacial acetic acid to the filtrate to produce a *p*H of 6.5. Precipitate the excess lead with hydrogen sulfide, filter, and add sufficient distilled water to the filtrate to make 250 ml. Preserve the soln under toluene in a refrigerator. Prepare a fresh soln at not more than 30-day intervals.

Salt soln A.—Dissolve 25 gm of monobasic potassium phosphate and 25 gm of dibasic potassium phosphate in sufficient distilled water to make 250 ml of soln.

Salt soln B.—Dissolve 10 gm of magnesium sulfate, 0.5 gm of reagent sodium chloride, 0.5 gm of ferrous sulfate, and 0.5 gm of manganese sulfate in sufficient distilled water to make 250 ml.

Stock culture of Lactobacillus casei.—To 10 ml of yeast extract soln in 90 ml of distilled water add 1 gm of anhydrous dextrose and 1.5 gm of agar, and heat the mixture on a steam bath until the agar has dissolved. Add approximately 10 ml portions of the hot soln to test tubes, plug the tubes with non-absorbent cotton, sterilize in an autoclave at 15 pounds' pressure (121.5°C.) for 20 min., and allow to cool in an

upright position. Prepare stab cultures in 3 or more of the tubes, using a pure culture of Lactobacillus casei,* incubate for 16 to 24 hours at any selected temperature between 30° and 37°C., but held constant to within ±0.5°C., and, finally, store in a refrigerator. Prepare a fresh stab of the stock culture every week, and do not use for inoculum if the culture is more than 2 weeks old.

Culture medium.—To each of a series of tubes containing 5 ml of the basal medium stock soln add 5 ml of distilled water containing 1 microgram of riboflavin. Sterilize in an autoclave at 15 pounds pressure (121.5°C.) for 20 min.

Inoculum.—Make a transfer of cells from the stock culture of Lactobacillus casei to a sterile tube containing 10 ml of culture medium. Incubate this culture for 16 to 24 hours at any selected temp. between 30° and 37°C., but held constant to within ±0.5°C. Under aseptic conditions centrifuge the culture and decant the supernatant liquid. The inoculum is prepared by suspending the cells from the culture in 10 ml of sterile isotonic soln of sodium chloride. If assays are to be made on each of several successive days, the inoculum may be prepared by successive daily transfer to the culture medium for a period not exceeding 1 week.

ASSAY PROCEDURE

Prepare standard riboflavin tubes as follows: To duplicate tubes, 16×150 mm in size, add 0.0 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, and 5.0 ml, respectively, of the standard riboflavin soln. To each of these tubes add 5 ml of basal medium stock soln and sufficient distilled water to bring the volume in each tube to 10 ml.

Prepare tubes containing the material to be assayed as follows: To duplicate tubes add, respectively, 0.5 ml, 1.0 ml, 1.5 ml, and 2.0 ml of the test soln of the material to be assayed. To each of these tubes add 5 ml of basal medium stock soln and sufficient distilled water to bring the volume in each tube to 10 ml.

After mixing thoroly, plug the tubes of the two series mentioned above with nonabsorbent cotton and autoclave at 15 pounds pressure (121.5°C.) for 20 min. Cool, aseptically inoculate each tube with 1 drop of inoculum, and incubate for 72 hours at any selected temperature between 30° and 37°C., but held constant to within ±0.5°C. Contamination of the assay tubes with any organism other than Lactobacillus casei invalidates the assay. Keep all of the tubes in darkness or semi-darkness during their preparation and incubation.

Transfer the contents of each tube to a suitable container, using approximately the same quantity of distilled water in each instance for rinsing. Titrate the contents of each flask with tenth-normal sodium hydroxide, using bromothymol blue as the indicator, or to a *p*H of 6.8 measured electrometrically.

CALCULATION

Prepare a standard curve of the riboflavin standard titrations by plotting the average of the titration values expressed in ml of tenth-normal sodium hydroxide for each level of the riboflavin standard soln used, against micrograms of riboflavin contained in the respective tubes. From this standard curve determine by interpolation the riboflavin content of the test soln in each duplicate set of tubes. Discard any values which show more than 0.25 or less than 0.05 microgram of riboflavin in each tube. Calculate the riboflavin content in each ml of test soln for each of the duplicate sets of tubes. The riboflavin content of the test material is calculated from the average of the values obtained from not less than three sets of these tubes which do not vary by more than ±10 per cent from the average. If the titration values of two or more of the duplicate sets of tubes containing the test soln fall be-

* Pure cultures of Lactobacillus casei may be obtained from the American Type Culture collections, Georgetown University Medical School, Washington, D. C., as number 7469.

low the titration values of the riboflavin standard tubes containing 0.05 to 0.25 microgram of riboflavin, the riboflavin content of the test soln is too low to permit calculation of riboflavin content of the test material. Titration values exceeding 2 ml for the tubes of the standard riboflavin soln series containing 0.0 ml of the soln indicate the presence of an excessive amount of riboflavin in the basal medium stock soln and invalidate the assay.

(3) The following method for nicotinic acid was adopted as official, first action:

1945 U.S.P.-A.O.A.C. COLLABORATIVE ASSAY METHOD FOR NICOTINIC ACID

Test soln of the material to be assayed.—Place an accurately weighed quantity of the material to be assayed, sufficient to represent approximately 0.1 mg of nicotinic acid, in a 300 ml flask, add 100 ml of normal sulfuric acid, and mix thoroly. Heat the mixture in an autoclave at 15 pounds pressure (121.5°C.) for 30 min., cool, add 1 *N* sodium hydroxide to produce a *p*H of 6.8, and add sufficient distilled water to make 1000 ml.

Standard nicotinic acid soln.—Dissolve an accurately weighed 50 mg portion of U.S.P. Nicotinic Acid Reference Standard in alcohol, and add sufficient alcohol to make 500 ml. Store this stock soln in a refrigerator. Prepare the Standard Soln by diluting 1 ml of the stock soln, which has been warmed to room temp., with sufficient distilled water to make 1000 ml, representing 0.1 microgram of the Reference Standard in each ml of soln. Prepare fresh Standard Soln for each assay.

Basal Medium Stock Soln

Acid-hydrolyzed casein soln	25 ml
Cystine-tryptophane soln	25 ml
Dextrose anhydrous	10 ml
Sodium acetate anhydrous	5 gm
Adenine-guanine-uracil soln	5 ml
Riboflavin-thiamine-biotin soln	5 ml
p-Aminobenzoic acid-calcium pantothenate-pyridoxine soln	5 ml
Salt soln A	5 ml
Salt soln B	5 ml

Mix the ingredients, adjust the soln to a *p*H of 6.8, and add sufficient distilled water to make 250 ml.

Acid-hydrolyzed casein soln.—Mix 100 gm of vitamin-free casein with 500 ml of constant-boiling hydrochloric acid (approximately 20 per cent HCl) and reflux the mixture for 24 hours. Remove the hydrochloric acid from the mixture by distillation under reduced pressure until a thick paste remains. Redissolve the resulting paste in distilled water, adjust the soln to a *p*H of 3.5 ($\pm$0.1) with 1 *N* sodium hydroxide and add sufficient distilled water to make 1000 ml. Add to the soln 20 gm of activated charcoal, and stir for 1 hour, then filter. Repeat the treatment with activated charcoal if the filtrate does not appear straw colored to colorless. Store this soln under toluene in a refrigerator. Filter the soln if a precipitate forms on storage.

Cystine-tryptophane soln.—Suspend 4 gm of l-cystine and 1 gm of l-tryptophane (or 2 gm of d, l-tryptophane) in 700–800 ml of distilled water, heat to 70–80°C., and add 20 per cent hydrochloric acid dropwise with stirring until the solids are dissolved. Cool to room temp. and add sufficient distilled water to make 1000 ml. Store the soln in a refrigerator.

Adenine-guanine-uracil soln.—Dissolve 0.1 gm each of adenine sulfate, guanine hydrochloride, and uracil with the aid of heat in 5 ml of 20 per cent hydrochloric acid, and add sufficient distilled water to make 100 ml. Store the soln in a refrigerator.

Riboflavin-thiamine hydrochloride-biotin soln.—Prepare a soln containing in each ml 20 micrograms of riboflavin, 10 micrograms of thiamine hydrochloride, and 0.04 microgram of biotin by dissolving crystalline riboflavin, crystalline thiamine hydrochloride, and crystalline biotin (free acid) in fiftieth normal acetic acid. Store the soln, protected from light, under toluene in a refrigerator.

p-Aminobenzoic acid-calcium pantothenate-pyridoxine hydrochloride soln.—Prepare a soln in neutral 25 per cent alcohol to contain 10 micrograms of p-aminobenzoic acid, 20 micrograms of calcium pantothenate, and 40 micrograms of pyridoxine hydrochloride in each ml. Store the soln in a refrigerator.

Salt soln A.—Dissolve 25 gm of monobasic potassium phosphate and 25 gm of dibasic potassium phosphate in sufficient distilled water to make 500 ml of soln. Add 5 drops of conc. hydrochloric acid and store under toluene.

Salt soln B.—Dissolve 10 gm of magnesium sulfate heptahydrate, 0.5 gm of reagent sodium chloride, 0.5 gm of ferrous sulfate heptahydrate, and 0.5 gm of manganese sulfate monohydrate, in sufficient distilled water to make 500 ml. Add 5 drops of conc. hydrochloric acid and store under toluene.

Stock culture of Lactobacillus arabinosus 17.5.—Dissolve 2 gm of yeast extract in 100 ml of distilled water, add 0.5 gm of anhydrous dextrose, 0.5 gm of anhydrous sodium acetate, and 1.5 gm of agar, and heat the mixture on a steam bath until the agar has dissolved. Add approximately 10 ml portions of the hot soln to test tubes, plug the tubes with non-absorbent cotton, sterilize in an autoclave at 121.5°C. for 20 min., and allow to cool in an upright position. Prepare stab cultures in 3 or more of the tubes, using a pure culture of Lactobacillus arabinosus 17-5,* incubate for 16–24 hours at any selected temp. between 30° and 37°C., but held constant to within ±0.5°C., and finally store in a refrigerator. Prepare a fresh stab of the stock culture every week and do not use for inoculum if the culture is more than 1 week old.

Culture medium.—To each of a series of tubes containing 5 ml of the basal medium stock soln add 5 ml of distilled water containing 1 microgram of nicotinic acid. Sterilize in an autoclave at 121.5°C. for 20 min.

Inoculum.—Make a transfer of cells from the stock culture of *Lactobacillus arabinosus 17.5* to a sterile tube containing 10 ml of culture medium. Incubate this culture for 16–24 hours at any selected temp. between 30° and 37°C., but held constant to within ±0.5°C. The cell suspension so obtained is the inoculum.

Assay procedure.—Prepare standard nicotinic acid tubes as follows: To duplicate test tubes, add 0.0 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml, 4.0 ml, 4.5 ml, and 5.0 ml, respectively, of the standard nicotinic acid soln. To each of these tubes add 5 ml of basal medium stock soln and sufficient distilled water to bring the volume in each tube to 10 ml.

Prepare tubes containing the material to be assayed as follows: To duplicate tubes add, respectively 1.0 ml, 2.0 ml, 3.0 ml, and 4.0 ml of the test soln of the material to be assayed. To each of these tubes add 5 ml of basal medium stock soln and sufficient distilled water to bring the volume in each tube to 10 ml.

After thoro mixing plug the tubes of the two series mentioned above with non-absorbent cotton, and autoclave at 121.5°C. for 15 min. Cool, aseptically inoculate each tube with 1 drop of inoculum, and incubate for 72 hours at any selected temp. between 30° and 37°C., but held constant to within ±0.5°C. Contamination of the assay tubes with any organism other than *Lactobacillus arabinosus* invalidates the assay.

Titrate the contents of each test tube with tenth-normal sodium hydroxide, using bromothymol blue as the indicator, or to a *p*H of 6.8 measured electrometrically.

* Pure cultures of *Lactobacillus arabinosus 17.5* may be obtained from the American Type Culture Collection, Georgetown University Medical School, Washington, D. C., as number 8014.

Calculation.—Prepare a standard curve of the nicotinic acid standard titrations by plotting the average of the titration values expressed in ml of tenth-normal sodium hydroxide for each level of nicotinic acid standard soln used, against micrograms of nicotinic acid contained in the respective tubes. From this standard curve determine by interpolation the nicotinic acid content of the test soln in each tube. Discard any values which show more than 0.4 or less than 0.05 microgram of nicotinic acid in each tube. Calculate the nicotinic acid content in each ml of test soln for each of the tubes. The nicotinic acid content of the test material is calculated from the average of the values obtained from not less than 6 of these tubes which do not vary by more than ±10 per cent from the average. If the titration values of two or more of the tubes containing the test soln fall below the titration values of the Nicotinic Acid Standard tubes containing 0.05 to 0.4 microgram of nicotinic acid, the nicotinic acid content of the test soln is too low to permit calculation of nicotinic acid content of the test material. Titration values exceeding 2 ml for the tubes of the standard nicotinic acid soln series containing 0.0 cc of the soln indicate the presence of an excessive amount of nicotinic acid in the basal medium stock soln and invalidate the assay.

(4) The official, first action, method for carotene, sections **36.7** and **36.8** (p. 600) for hay and dried plants, and the tentative method for carotene, sections **36.7–36.15,** inclusive, were deleted.

(5) The following method for carotene was adopted as tentative.

CAROTENE

Extraction of Pigments

REAGENTS

(1) *Alcoholic potassium hydroxide.*—Dissolve 12 gm of KOH in 100 ml. of 95% ethyl alcohol.

(2) *Petroleum ether.*—Use petroleum ether or Skellysolve. B. P. 30° to 70°C.

(3) *Adsorbent.*—Use $MgCO_3$, $CaHOP_4$, or other adsorbents which will adsorb less than 5% carotene when tested by the following method: Place approximately 2 gm of the adsorbent in a tube as described below and pass thru it 50 ml of a soln of purified carotene in petroleum ether containing 1.0 to 1.5 micrograms of carotene per ml. Wash the column with petroleum ether and determine the carotene in the filtrate. If the adsorbent is too retentive of carotene, another lot should be tried. If later in the analyses the adsorbent is found not to have high enough adsorptive power to separate the xanthophylls and other impurities from the carotene mix with it a small amount of MgO (ca 2½%). The amount of MgO will depend on the adsorptive power of the carbonate and of the oxide.

(4) *Purified carotene soln.*—Dissolve about 0.1 gm crystalline carotene (90% beta, 10% alpha) in about 2–3 ml of chloroform. Add 25 ml methanol and filter. Wash the crystals of carotene once with a few ml methanol. Dry in a vacuum over anhydrous $CaCl_2$ for not more than 1 hour. Dissolve 20 mg of the purified carotene in about 2 ml of chloroform and dilute to 100 ml with petroleum ether. This stock soln contains 200 micrograms per ml of carotene.

(A) Extraction of Materials (dried hays, grasses, etc.) Other than Yellow Corn, Sweet Potatoes, and Carrots

Reflux 1–5 gram sample for 30 min. with 20–100 ml of 12% alcoholic KOH. If portions of the sample collect on the sides of the flask wash down with alcohol. After

refluxing, cool the contents of the flask, add 50 ml of petroleum ether, shake for a minute, and allow the sediment to settle. Decant the liquid into a 500 ml separatory funnel. Repeat extraction twice more with 25 ml portions of petroleum ether. Break up the residue, which sometimes forms an adherent mass, by shaking with 10–15 ml of 95% ethyl alcohol. Make 2 or 3 additional extractions with 25 ml portions of petroleum ether, and continue until the petroleum ether is colorless.

Pour gently about 100 ml of distilled water thru the alcohol-petroleum ether soln into a separatory funnel. Draw off the alkaline alcohol-water soln and extract one more time by shaking with a 30 ml portion of petroleum ether in another funnel. Any emulsion may be cleared by adding 1 ml of ethyl alcohol. Combine the petroleum ether extracts and wash them 5 times with 50 ml portions of distilled water. Concentrate if necessary, filter over anhydrous Na_2SO_4 and make up to a volume of 100 or 200 ml.

(*B*) *Extraction of Dehydrated Carrots and Sweet Potatoes*

Preparation of sample for extraction.—(a) To 1 or 2 grams of finely ground sample add approximately 4 or 8 ml of distilled water and allow to stand 30 min. (b) If the dehydrated material contains considerable moisture and is consequently too tough to grind in a Wiley or other type of mill, soak 5 gm of the unground material in 20 ml of water for 2 hours. Then grind in a mortar with a pestle.

Extraction of sample.—Add 50 to 100 ml of 95% ethanol and reflux 15 min. Filter the alcoholic soln thru a fritted glass funnel and wash the residue with small portions of ethanol. Remove the residue, add 25 ml of 95% ethanol, and reflux again for 15 min. Filter and wash as above. If the residue contains more than a very slight amount of yellow color, reflux a third time. To the combined alcoholic solns and washings add 50 to 100 ml petroleum ether. Shake, and then add 100–150 ml of water and allow the layers to separate. Draw off the aqueous-alcoholic layer and re-extract with 25–50 ml of petroleum ether. Repeat this extraction until the petroleum ether layer separates colorless, or only very slightly colored. Combine the petroleum ether extracts and wash 5 times with 50 ml portions of distilled water. Concentrate if necessary, filter over anhydrous sodium sulphate and make up to a volume of 100 or 200 ml.

Purification of Pigments

Prepare a column as follows: Place about 2 gm of adsorbent in a glass tube about 10 mm wide and about 15 cm tall, constricted at one end and plugged with a wad of cotton. Apply suction and pack the magnesium carbonate, firmly but not too tightly with a cork having a smooth surface and attached to a glass rod. Concentrate to about 10 ml, *in vacuo*, a 50 ml aliquot of the carotene soln obtained above. Place a few ml of petroleum ether on the column of adsorbent, apply suction, and before the petroleum ether is drawn in, place the concentrated aliquot in the tube. Allow all the extract to be very nearly drawn into the column and then add petroleum ether. Always keep the surface of the column covered with petroleum ether. Wash the column with petroleum ether until the pure carotene is washed thru. Collect in a suitable container and dilute to 50 ml.

Determination of Carotene in Extracts

Determine the carotene in the extract with a photoelectric colorimeter or a spectrophotometer.

(a) *Photoelectric colorimetric method.*—The photoelectric colorimeter with the proper filter (440) must first be calibrated against pure carotene. Purify crystalline carotene as given above, and make up a stock soln containing 200 micrograms per

ml of carotene. From this soln make up solns contains 2.0, 1.6, 1.2, 0.8, 0.4, and 0.2 micrograms of carotene per ml, and obtain the colorimeter reading for these solns. Plot colorimeter readings against concentration of carotene on arithmetical graph paper. Use this curve for estimating carotene in the sample. Photoelectric colorimeters may vary in their sensitivity and there should be some way of checking them. To do this, make up a potassium dichromate soln containing 0.02% potassium dichromate. Adjust instruments to always give the same reading for this soln. If the instrument cannot be adjusted to give the desired reading, recalibrate the instrument.

After the photoelectric colorimeter has been calibrated, read the color of the carotene solutions and calculate the micrograms of carotene per gm of sample.

(b) *Spectrophotometric method.*—Read the optical density at 450 millimicrons and calculate the micrograms of carotene per gram of sample by use of the formula

$$M = \frac{10{,}000\ DV}{Edg}$$

where M is the micrograms of carotene per gm of sample, D the density of adsorption, V the ml of soln, E the adsorption coefficient, 2580, d the depth of soln, and g the grams of sample.

37. WATERS, BRINE, AND SALT

No additions, deletions, or other changes.

38. RADIOACTIVITY

No additions, deletions, or other changes.

39. DRUGS

(1) The following method was tentatively adopted for quinine ethylcarbonate:

Weigh a quantity of the powdered material equivalent to about one grain of the alkaloid and transfer to a 125 ml Erlenmeyer flask. Add 5 ml of alcohol and 15 ml of 0.5 *N* NaOH and place a small funnel in neck of flask. Heat on steam bath for 10 min. Transfer the soln to a separatory funnel. Rinse the hydrolysis flask with small portions of water followed by small portions of dilute hydrochloric acid and add rinsings to the soln in the separatory funnel. Acidify soln with dilute hydrochloric acid and then render alkaline with NH_4OH. Extract with four 25 ml portions of $CHCl_3$. Wash combined chloroform extracts with 5 ml of water and filter thru a pledget of cotton into a weighed beaker. Evaporate to dryness on steam bath, add 5 ml of alcohol, and evaporate the alcohol. Dry at 100°C. for one hour and weigh. Determine quinine by the method given in *Methods of Analysis, A.O.A.C.*, 6th edition, section **39.13**, beginning with " . . . dissolve the amorphous alkaloid . . . . " One ml of 0.02 *N* H_2SO_4=0.00649 g of anhydrous quinine ($C_{20}H_{24}O_2N_2$).

(2) The tentative method for prostigmine, section **39.87** (p. 693), was adopted as official, first action.

(3) The following method for plasmochine was tentatively adopted:

Weigh a powdered sample equivalent to about 0.02 gm Plasmochin Napthoate and transfer to a 100 ml beaker. Add 10 ml water, 5 ml of 10% hydrochloric acid and stir until powder is thoroly wet. Add 20 ml of water and stir again. Filter thru a

Gooch or Selas crucible, washing with several portions of water until the filtrate becomes colorless.

Transfer the filtrate completely to a separatory funnel, make alkaline with ammonia, and extract with several portions of chloroform. A test for complete extraction can be made by evaporating a separate extract to dryness whereupon the addition of N/1 acid should not produce a yellow color. If positive, this can be returned to the separatory funnel and reextracted. Wash the chloroform extracts with several ml of water, filter thru cotton, evaporate to about 5 ml, then add 25 ml N/50 acid. Continue the heating and when the bulk of the chloroform has evaporated, stir with a glass rod to facilitate the removal of the last droplets of chloroform in order to avoid prolonged heating of the soln. Cool and titrate with N/50 alkali using bromocresol purple as indicator. Titrate to a purple tint.

1 ml N/50 acid = 0.01406 gm Plasmochin Naphthoate
1 ml N/50 acid = 0.0063 gm Plasmochin base

(4) The following methods for 8-hydroxyquinoline sulphate were tentatively adopted:

METHOD I

(For amounts of hydroxyquinoline sulfate between 25 and 250 mg. Use this method wherever nature of sample permits.)

REAGENTS

(a) *Bromide-bromate soln.*—0.1 *N* Br. (See *Methods of Analysis*, 6th Ed. 1945, **39.28(c)**, p. 674.

(b) *Methyl red soln.*—See id., **43.10(c)**, p. 805.

EXTRACTION

(a) If no interfering substances are present, dissolve the sample in about 75 ml of water and add 5 ml of HCl.

(b) *Non-oily preparations.*—Extract preferably from a soln alkaline with sodium bicarbonate or borax. If extraction from such a medium is impracticable, or if compounds of ammonia or heavy or alkaline earth metals are present, add methyl red soln and adjust with sodium hydroxide and/or hydrochloric acid to barely acid. Add sodium acetate in the proportion of 1 gm per 100 ml of soln. If heavy or alkaline earth metals are present, add also 2 ml glacial acetic acid per 100 ml of scln.

Extract the solution, adjusted by any of these procedures, with sufficient 20 ml portions of chloroform. For an alkaline or barely acid soln, generally 6 extractions suffice. With extra acetic acid added, 10 to 12 extractions are needed. A test for complete extraction may be made by adding a little HCl (1+9) to the last portion, evaporating the chloroform on the steam bath, adjusting to 70°C., adding a drop of 0.01 *N* Br and then a drop of diluted methyl red, which should be bleached immediately.

Extract the combined chloroform extracts with 5 portions of HCl (1+9). If salicylic acid, volatile oils, etc., are present, wash each portion of acid with the same 10 ml of ether. If the sample contained phenol or other volatile, interfering substances and these have not been completely removed by the preceding process, boil the acid soln to remove them, maintianing the volume approximately constant by adding more water.

(c) *Ointments, etc.*—Transfer the sample to a separator with 50 ml ether, extract with five 10 ml portions of HCl (1+9). If salicylic acid, etc., are present, wash each acid portion with the same 10 ml of ether. Add methyl red and make just alkaline

with 10% NaOH, then just acid with dilute HCl. Adjust the *p*H and proceed as under (b) commencing at "Extract the soln."

Titration.—Adjust the acid soln obtained under (a), (b) or (c) to 50°C. and maintain it at this temperature during the titration by occasionally reheating. Add a drop (or more) of methyl red solution from a buret and titrate with 0.1 *N* Br. As this is added, the color of the liquid gradually changes from brown orange to yellow. Add more indicator whenever the yellow is about reached. At slightly beyond the halfway point, dibromhydroxyquinoline may crystallize out and absorb dye. Disregard the color of the precipitate; judge by that of the soln. (By diluting to not over 0.1 gm hydroxyquinoline sulfate per 100 ml, the formation of the precipitate can be avoided.) The end point is reached when, after waiting 10 seconds for the absorption of the last drop of Br and adding a drop of indicator, it is bleached almost immediately. The timing for the addition of the drop of indicator at the end point is important, as the proper conditions prevail for but a brief period.

Read the volumes consumed of the two solutions. Measure 10 ml of the methyl red soln into an Erlenmeyer flask, add 2 ml HCl and titrate with 0.1 *N* Br. Correct the main titration for the amount of Br consumed by the volume of indicator used.

1 ml 0.1 *N* Br = .005078 gm $(C_9H_7NO)_2 \cdot H_2SO_4 \cdot H_2O$

METHOD II

(For amounts between 2 and 10 mg)

REAGENTS

(c) *Bromide-bromate soln.*—0.01 *N* Br. Dilute one volume of (a) accurately to ten volumes.

(d) *Diluted methyl red soln.*—Dilute 1 volume of (b) to 4 volumes with water and sufficient NaOH to dissolve the dye.

Extract as in Method I. Start the titration as in Method I, using the 0.01 *N* Br and diluted methyl red soln, instead of the stronger reagents. Use as little indicator as possible. When near the end point, evidenced by more rapid consumption of indicator, heat to 70°C., and complete the titration at this temperature.

(5) The following methods for metrazole were tentatively adopted:

METHOD I

By means of a pipet transfer 5 ml of the soln to a separator, add 3.5 gm of ammonium sulfate and a drop of ammonium hydroxide soln and shake the soln four times with chloroform for about one minute each time and using 50, 30, 20, and 10 ml portions. To insure complete extraction shake the soln a fifth time with 10 ml of chloroform and evaporate the solvent separately with the precautions described below. Filter each portion of chloroform thru cotton into a tared beaker or Erlenmeyer flask. Evaporate or distill about three-fourths of the solvent and evaporate the remainder to apparent dryness using a gentle current of air. Add 5 ml of anhydrous ether to the residue and allow to evaporate spontaneously. Repeat the treatment with anhydrous ether. Dry the residue over sulfuric acid to constant weight.

METHOD II

Weigh sufficient of the powdered material to yield from 0.1 to 0.2 gm of metrazole, transfer to a separator, add 4 g of ammonium sulfate and 5 ml of water, and proceed as for metrazole soln. Method I, beginning with the expression "a drop of ammonium hydroxide soln."

(6) The following method for phenolphthalein in bile salts was tentatively adopted:

Transfer 0.4 to 0.7 g of sample to a separator containing ca 10 ml of *N* NaOH. Shake to dissolve the sample, acidify with HCl and extract with $CHCl_3$-ether (3+1). Pass each portion of solvent thru a separator containing 15 ml of freshly prepared saturated soln of $NaHCO_3$, then thru a separator containing 15 ml of H_2O. Transfer the solvent to a beaker without filtering and evaporate to dryness on steam bath with aid of fan. Proceed as directed in **39.170** (*Methods of Analysis*, 6th ed.). Report per cent of phenolphthalein in the sample.

40. MICROBIOLOGICAL METHODS

No additions, deletions, or other changes.

41. MICROCHEMICAL METHODS

No additions, deletions, or other changes.

42. EXTRANEOUS MATERIALS IN FOODS AND DRUGS

(1) Additions and revisions in Chapter 42 were tentatively adopted for the several sections, as follows:

42.1 (a) Add to end of paragraph:

"This recess should be filled with a water-insoluble glue or cement."

(b) Add "or bolting cloth" after word "papers" in sentence beginning "Use Hirsch funnels . . . . "

Add to end of first paragraph:

"For use of Büchner funnel see **(g)** below."

42.1 (i) Add the following sentence:

"Where the use of a No. 100 sieve is specified for filth recovery by suction in a Büchner funnel, use can be made of a No. 100 sieve soldered to the base of a metal ring ca 5 cm high that will fit into the Büchner funnel."

42.1 (i) Add the following as separate paragraphs:

"*Silk bolting cloths.*—Where the disks of silk bolting cloths are to be used, these disks are best prepared by boiling the large squares of silk before cutting them into circles. Circles cut from unboiled silk quickly shrink and become misshapen. By first boiling the silk the circles will remain the approximate size they are cut. Rulings should be applied after boiling but before cutting, and can be made with India ink, blue-black ink, or indelible pencil.

"Silk bolting cloth disks can be bleached by soaking overnight in 3% H_2O_2 and then washing them in H_2O, or by boiling in an alkaline H_2O_2 soln prepared by adding 10 ml of 2.5% NaOH and 5 ml of 30% H_2O_2 to a liter of H_2O."

42.1 (i) "*Maintaining heat in funnel during filtration.*—The filtering funnel and its contents may be kept hot during filtration by means of a light bulb with reflector held over the funnel. The degree of heat may be controlled by varying bulb size or by raising or lowering the light bulb and reflector."

42.1 (i) "*Butter stirrer.*"—See **22.110.**

42.2 Add after end of first sentence: "Hot H_2O is ca 50°."

42.2 (c) Substitute following paragraphs for paragraph (c):

"*Special reagent. Pancreatin solution.*—For each 50–225 g portion of sample use the filtered extract from 5 g pancreatin (U.S.P.) prepared as follows: Mix 5 g pancreatin in 50 ml H_2O. Allow to stand about one-half hour or continue mixing in a mechanical mixer for 5–15 min. (If a large amount of foam is present, it can be

broken by repeatedly applying and breaking a vacuum.) Centrifuge to remove most of the coarse suspended material (ca 2500 r.p.m. for ca 15 min.) and then decant thru a loose moistened cup of absorbent cotton in a 60° funnel. In filtering a liter of pancreatin soln use about 4 inches of cotton in a 4–5 inch funnel. Smaller amounts can be handled proportionally. With suction, pass the filtrate from the cotton thru a rapid action filter paper in a Hirsch or Büchner funnel in such a manner that filth cannot pass around the edges of the paper. Add H_2O to reconstitute to 50 ml extract for each 5 g dry pancreatin used.

"When digesting with pancreatin, adhere to the following points: In neutralizing an acid soln prior to pancreatic digestion use NaOH soln until the mixture becomes ca neutral. Then complete the adjustment with Na_3PO_4 soln. Unless the consistency of the mixture prohibits such a procedure, correct the *p*H before adding pancreatin. When pancreatin is added to an uncorrected mixture, bring to the correct *p*H as soon as possible."

42.2 Add to end of section the following:

"(f) *Alcohol.*—Use 95% unless otherwise specified.

"(g) *Castor oil.*—Use U.S.P

"(h) *Kerosene or gasoline.*—Use interchangeably.

"(i) *Mineral oil.*—Use only when specified. It is not interchangeable with gasoline, kerosene, or castor oil.

"(j) *Hydrochloric acid.*—Per cent HCl is per cent hydrogen chloride in H_2O.

"(k) Mixtures of CCl_4 and petroleum ether adjusted to correct specific gravity may be substituted for $CHCl_3$."

42.4 (a) Add to end of second paragraph:

"Emulsions which have risen into the neck of the flask may be broken with alcohol, capryl alcohol, or ethyl ether."

42.4 (a) Delete the words "oil, or" in the 2d line, 4th paragraph.

42.4 (a) Add as 5th paragraph the following:

"Examine the residues in the flask for rodent pellets and other microscopic heavy filth."

Add as 6th paragraph the following:

"When the trapped-off material contains an appreciable amount of starch material, add sufficient conc. HCl to make 1–2% HCl, boil, and filter while hot."

Add as 7th paragraph the following:

"As an aid in rinsing material from fine mesh sieve, fit snugly a plastic or metal disk just underneath sieve, and rinse with stream of H_2O from wash bottle."

42.4(b) (3) Change "into" in third line to "in."

42.4 (b) (5) Change "filth," line 4, to "filter."

42.4 (b) Add as No. (6) the following paragraph:

"Residues of chocolate on filter papers can be bleached with hypochlorite soln. Note, however, that hypochlorite swells and gradually dissolves hairs."

42.4 (c) Add as (d) the following paragraph, after (c):

"At regular intervals run determinations on solns used and glassware."

42.7 Add after "trap-off" near end of paragraph: "Filter and examine." Strike out rest of paragraph.

42.10 Add after word "Sample" 1st line:

"of cocoa or cocoa substitute, or 50 g sample of coffee or coffee substitute, in which case double the amount of $CHCl_3$ and size of beaker and trap flask."

Add after sentence ending in "mouth of flask" "and add H_2O to maintain volume."

Change title by deleting "and" and adding "coffee and coffee substitutes."

DAIRY PRODUCTS

42.11 Add "HCl" to list of reagents given in 2d sentence of paragraph.

Add following as 2d paragraph:

"*Alternative procedure.* Substitute fritted glass filter, either Pyrex fine or Jena IG4 in place of Gooch and casserole in all operations except in boiling."

CHEESE PRODUCTS

42.12 Add to end of first paragraph after striking out period:

"and hot H_2O (ca 60°) for cold H_2O. In all cases except as noted use paper for filtration. When filtration on paper cannot be accomplished, use a No. 100 sieve and transfer residue to paper. For cheddar cheese, **42.12(b)** is the method of choice and for hard, skim, or grated cheese, **42.13(b)**.

After "proper temp." add "and also of stirring mixture similar to that in **22.110**."

42.12 (a) Add to end of paragraph:

"For Bondost cheese follow method up to filtration but pass material thru a No. 60 sieve and then thru 8XX cloth. Transfer residue to 400 ml beaker with hot H_2O. Add HCl to make concentration of 3–4% HCl, boil 15 min., transfer to 1-liter flask and trap in usual manner."

42.12 (b) Replace last sentence with the following:

"Stir ca 5–15 min. with continual boiling and filter thru paper. For blue cheese filter successively thru a No. 60 sieve, 6XX cloth, and 10XX cloth. Transfer residue on sieve to paper. Examine cloth as is."

42.13 (b) Add after "1 hour," the words "or longer." Add to end of paragraph:

"In some cases the addition of alcohol may not be necessary. Filter after boiling vigorously 15 min."

DRIED MILK

42.15 Change sentence in middle of paragraph beginning "Heat to 35–45° . . . " to read:

"Heat to 35–45° for 30 min. or until digested but adjust *p*H after 15 min. and 30 min. digestion to *p*H 2 with HCl." Strike out sentence beginning "Adjust to *p*H 2 . . . . "

Add the following method:

"Alternative Method. Filtration on Paper."

"(a) *For spray dried products.*—(Note: Under the low-power binocular microscope and against a dark background spray dried milk appears as minute hollow globules. Roller dried milk appears as irregular shaped flakes and granules.)

"To 1.5 liters warm H_2O (50–60°) in a 2-liter beaker, add 100 g milk powder while mixing with paddle type stirrer, **22.109**. Continue stirring until all powder has been wet and dispersed, washing down the sides of beaker with H_2O. Add 20 ml of conc phosphoric acid while stirring continuously followed by ca 0.5 ml caprylic alcohol to reduce foam. Rinse stirrer and filter soln thru paper (S & S No. 604, or Whatman No. 41 is recommended, but S & S Shark Skin may be used, with the ruled side down). Rinse beaker with warm H_2O and with alcohol. Before examining papers, wet liberally with H_2O."

"(b) *For roller dried products.*—Prepare an acid soln as above, using 100 g milk powder and same quantities of the other reagents. After adding acid and caprylic alcohol, add 20 ml of a 10% pepsin soln previously prepared by dissolving pepsin (NF granules or powder) in warm H_2O (40–50°) and filtering thru paper. Mix well, cover beaker with watch glass, and digest on steam bath for 30 min. Filter, and rinse beaker as above. If appreciable amounts of charred milk particles are present, use two or three filter papers. If paper clogs while filtering, add 1–2 ml phosphoric acid to the filter funnel followed by an excess of H_2O."

CREAM

42.17 (a) Substitute the following procedure for **42.17 (a)**:

"*For most creams.*—Place 1 pint of cream in a beaker or pan of convenient size, about 2 quart, and add ca 1 pint of hot H_2O (70–90°). More or less H_2O may be added so that the mixture ready for filtration will be 45–60°.

"Remove whole flies or other large filth particles which would be broken up by the stirrer. Place these on the sediment pad when completed.

"Place the pan under a malted milk stirrer and add while stirring sufficient 40% Calgon[2] soln to make the mixture slightly alkaline to red litmus. Use not less than 25 ml; an excess will not interfere with filtration. Stir 30 to 60 seconds or until curd is broken up. Filter with vacuum thru a sediment pad. If the pad clogs, filter the remaining portion thru a fresh disk. Rinse pan and funnel onto the sediment pad with the hot H_2O. Clogged pads indicate moldy cream in most cases; previous neutralization of cream with lime may result in clogged pads.

"Use sediment pads waffle side up, moisten with H_2O before adding cream mixture, and apply vacuum as the cream mixture is being poured down the sides of the funnel. Pour the cream into the funnel rapidly so that all of the filtering surface of the pad is covered. Do not pour cream mixture, rinsings, or stream from wash bottle directly against the pad surface."

42.17 (b) Add after "filtered" in 1st line the words "by **(a)**." Add following method to this section:

"*Alternative method.*—Place sample jars in H_2O bath at approximately 60° to pre-heat, thus shortening the later heating time. (Quart Mason fruit jars with 1 pint calibration and ground spot for number, and 2-piece metal ball enclosure are suggested. A wash boiler makes a convenient H_2O bath for use in creameries.) Pour sample into top pan of porcelain double boiler, rinsing sample jar with a minimum amount of H_2O. Heat to 60°. Place under mixer and stir while adding 10% NaOH soln to faint pink to phenolphthalein. Add approximately 30 ml 40% Na hexametaphosphate soln, with continued stirring. Filter thru sediment disk. After all cream has passed thru pad, rinse boiler and funnel with small amount of hot H_2O from wash bottle. A small amount of alcohol, isopropyl or ethyl, is often beneficial at this stage to break and rinse down the foam. If the pad clogs, transfer the mixture from the funnel back to pan, add 30 ml more of Na hexametaphosphate soln, heat to 55° if necessary, stir a moment and filter thru a fresh pad. The sediment on the clogged pad may be transferred to other pad by a stream of H_2O from wash bottle. In cases where the pad clogs quickly, the cream may be transferred to original sample jar, digested with pancreatin, heated to near boiling if necessary, and filtered as before."

BUTTER

42.21 (a) Change sentence in middle of paragraph beginning "Filter at once thru," to read "Filter at once."

42.21 (b) Add the following method to this section:

"*Alternative method. Filtration on sediment pad.*—Weigh 225 g butter into a liter beaker, add 250 ml hot H_2O and 20 ml of 85% phosphoric acid and bring to boil, stirring continuously. Then filter with suction thru a 1¼ inch Johnson sediment pad. Wash with hot H_2O.

SHELLED NUTS

42.22 Add the following procedures:

"*Light filth. Alcohol flotation.*—Place 100 g of sample in a 1-liter Wildman trap flask. Add 10 ml of 10% Na oleate soln plus 240 ml of 40% alcohol. Heat to boiling,

[2] Calgon, Adjusted. Manufactured by Calgon, Inc., Pittsburgh, Pa.

then cool below 20°. Add 25 ml gasoline, mix thoroly, and let stand 5 min. Fill the flask with 40% alcohol and let stand 30 min., stirring occasionally. Trap off, filter, and examine. Add 20 ml gasoline to trap flask, stir vigorously and then let stand 10 min. before trapping off. If this yields appreciable filth, decant most of the liquid from the trap flask, add 20 ml gasoline, stir, fill with H_2O, let stand 10 min., and trap off again. Pour the material from the trap flask onto a No. 40 sieve. Transfer the residue to a white-enameled pan and examine for gross filth."

"*Alternative procedure. Heavy and light filth.*—Thoroly mix each subdivision of the sample and weigh out 100-g portions from each sub into 400-ml beakers. Cover the nuts with petroleum ether, cover the beaker and boil under a hood for 30 min. Add sufficient ether to maintain the original volume. Decant the ether thru a smooth 7-cm filter paper in a Büchner funnel. Add ca 200 ml chloroform to the beaker and allow to settle 10–15 min. Remove the floating kernels with a spoon to the above-mentioned filter. Decant the chloroform thru the filter paper and dry the nuts by suction. Transfer the residue, if any, from the beaker to an ashless filter paper, and examine for heavy filth. Ignite and weigh to determine the sand and dirt. Transfer the nuts from the Büchner funnel to a 2-liter Wildman trap flask.

"Add 400 ml alcohol, cover, and boil for 20 min. maintaining the original volume with alcohol. Add ca 200 ml hot H_2O in small amounts, stir, and boil 10 min. Cool below 20°. Add 35 ml gasoline and mix thoroly. Allow to stand 5 min., fill with H_2O, and then allow to stand for 30 min., stirring occasionally. Trap off, filter, and examine.

"Add 20 ml gasoline to trap flask, stir vigorously and then allow to stand 10 min. before trapping off. If this yields appreciable filth, decant most of the liquid from the trap flask, add 20 ml gasoline, stir, fill with H_2O, allow to stand 10 min., and trap off again. Pour the material from the trap flask onto a No. 40 sieve. Transfer the residue to a white-enameled pan and examine for gross filth.

"Note: Curculio larvae cannot be extracted by gasoline flotations."

PEANUT BUTTER

42.24 After the phrase "100 mesh bolting cloth," line 6, add "or sieve."

BAKED PRODUCTS, PREPARED CEREALS

42.26 Add the following statement before the heading, "Preparation of Sample."

"In grossly contaminated samples or wherever obviously practicable, examine 'as is' with the naked eye. Use a Greenough type microscope when necessary. When the product is examined while whole, include an examination of the interior of the package for insects, webbing, and excreta."

42.27 After the first paragraph, but before the paragraph beginning, "(a) Fruit, nuts, and cocoanut absent," add a second paragraph as follows:

"Crusts or other tough pieces of bakery products that may remain relatively unaffected by the pancreatic digestion may be separated from the soft portion and digested by boiling in dilute HCl, not over 2%, and then treating this portion by the regular gasoline flotation method."

42.28 Change the method to read as follows:

"*Large Quantities of Bran or Chocolate Present*"

"*For most products.*—Pour thru a No. 140 sieve, and wash well with H_2O. Transfer the material on the sieve to a 1-liter Wildman trap flask, add ca 200 ml of 60% alcohol. Boil for 10–20 minutes, cool, add 40 ml gasoline. Stir and trap off using 60% alcohol. Filter and examine microscopically.

"*For chocolate covered wafers*—Centrifuge the entire product for ca 10 min. at ca 1400 r.p.m. in 250 ml (8 oz) centrifuge bottles. Pass the liquid, emulsion, and fat

layers above the sediment thru an 80-mesh bolting cloth with the aid of a stream of H_2O from a wash bottle. If only a small amount of debris remains on the cloth, examine microscopically; otherwise transfer the material retained on the cloth to a 2-liter trap flask. Add ca 100–125 ml hot H_2O to the centrifuge bottles, stopper, shake vigorously, and transfer to the trap flask. Cool to room temperature. Add 30 ml kerosene, mix, fill with H_2O, trap off, filter, and examine. (If a large amount of fine debris rises into the kerosene layer, pass the trapped-off material, first thru an 80-mesh cloth and then thru the filter paper. Examine the cloth and paper separately.)"

42.28 Change title beginning "Larger Quantities . . . " to:

"Alternative Procedure for Prod ...with Large Quantities of Bran or Chocolate Present."

WHITE WHEAT FLOUR

42.29 Delete the second paragraph.

42.30 Before the word "Transfer," line 6, add sentences as follows: "Add ca 10 ml gasoline to the material in the trap flask, stir gasoline into the mixture, and after ca 5 min. trap off into the same beaker."

42.30 Add the following statement:

"Screen the remaining contents of the first flask thru a No. 40 sieve. Wash the larger particles into a white-enameled pan to recover any rodent pellets or lumps of heavy filth which may have settled to the bottom of the flask."

42.30 Add the following two procedures:

"Sifting."—Sift 225 to 450 g of flour thru a No. 20 to No. 40 wire screen or bolting cloth. Count the insects retained on the sieve. In reporting results include a statement as to sieve size used. For this purpose, the insects may or may not be removed to a filter paper, glass plate, dish, etc., for counting.

"Optional for gross contamination."—Weigh 50 g of flour into a 250-ml beaker. (Larger samples may be used. In such instances the apparatus size and volume of reagent should be increased accordingly.) Add ca 100 ml $CHCl_3$ and stir. Pick out and count adult insects, larvae, and pupae that float to the surface."

42.31 Add to "(a) Optional for 1–4 samples" the following:

"Depending upon the size of the plate, larger amounts of flour can be used. Similarly a ruled glass plate can be used and the oil-flour mount covered with a glass cover. For example: use 0.5 g flour on the tomato rot count plate, **42.1(d)**. Weigh the flour in a counter-balanced scoop or directly to the plate. Thoroly saturate the flour on the counting plate, cover with the glass cover, and count insect excreta. To move or turn suspected particles, apply gentle pressure or move the cover slightly while observing thru the microscope."

Add the following new method under the title "White Wheat Flour" after **42.31.**:

"Insect Eggs in Flour"

"Transfer 50 g of flour to a 100-mesh sieve (if more than ca 0.1 g overs are obtained, a No. 60 or No. 80 sieve should be used to prevent slow filtration after digestion) and sift gently until no more flour passes thru. Transfer the portion remaining on the sieve to a 250-ml beaker. Wet the overs with 2–3 ml of alcohol. Add 30 ml of 5% H_2SO_4. Cover the beaker and heat on a steam bath for 10 min. Filter the contents thru a paper on a suction funnel using the minimum of suction necessary to filter. Keep the beaker partially inverted over the funnel and rinse with H_2O. Turn off the suction. Add 15–20 ml of ca N/10 iodine soln to the paper in the funnel. Allow ca 10–15 seconds for the iodine to stain the contents. Apply gentle

suction. After the iodine has passed thru the filter, wash the paper with 25–30 ml of 1% H_2SO_4, followed by several small H_2O washes. Transfer the paper to a Petri dish and examine at once under 20 magnification."

WHOLE CORN MEAL

42.32 After the phrase " . . . until very little corn tissue remains in beaker." (line 8) add "Be careful not to decant any rodent excreta fragments that may be present."

ALIMENTARY PASTES

42.37 Change **42.37** to read as follows:

"To 1000 ml of boiling 1% HCl add one-half pound paste. Continue heating for 30–40 min., or until the mixture becomes a finely divided mass that will not froth over when covered; cool somewhat. Neutralize with NaOH soln and bring to *p*H 7–8 with Na_3PO_4 soln. Cool to 35–40° and digest with pancreatin (**42.27**). Bring to a boil; cool; transfer to a 2-liter Wildman trap flask and extract and examine in the usual manner.

STARCH

42.38 Add the following new procedures after "**42.38,** Starch Filth."

"Germ Content of Wheat Germ Products"

"Weigh out 0.5 g of the well-mixed sample. Place small amounts of the weighed sample in a Petri dish and by means of a Greenough microscope and sharp-pointed forceps separate the germ from the other ingredients. Weigh bran and flour and calculate the percentage of germ."

"Filth in Brewer's Grits"

"(a) *Excreta.* Use **42.32.**

"(b) *Insect fragments and rodent hairs.* Use **42.33** substituting cold H_2O for the 60% alcohol."

"Soy Bean Flour for Filth"

"(a) *Light filth. Alcohol extraction.*—Add 300 ml of 40% alcohol to 50 g flour in a 1-liter Wildman trap flask. Heat to a vigorous boil with continuous slow agitation to prevent scorching. Cool to ca 20°, add 100 ml of 40% alcohol, then 25 ml kerosene, and mix thoroly. Fill with 40% alcohol and after 1 hour trap off and filter. Add 10 ml kerosene, stir down into the mixture and trap off again after 30 min. Clear the filter papers in mineral oil and examine at 20–30 ×.

"Pour the mixture in the trap flask thru a No. 40 or No. 50 sieve using H_2O as an aid in washing the flour thru the sieve. Rinse the residue on the sieve to a filter paper or Petri dish and examine for larvae, webbing, and rodent excreta.

"(b) *Alternative procedure. Hydrochloric acid digestion.*—Transfer 50 g of soy flour to a 2-liter Wildman trap flask. Add 300 ml of H_2O and 15 ml of concentrated HCl. Place the flask on a hot plate and bring the contents to boiling. Stir constantly to prevent the soy flour from sticking to the bottom of the flask and scorching. Considerable frothing occurs as the temperature approaches the boiling point. Transfer the flask to a H_2O bath and cool to room temperature. Add 20 ml of gasoline and mix thoroly. Allow to stand several minutes. Fill with H_2O. Stir several times during 20 min. Add several ml of alcohol into the gsaoline layer to aid in breaking any emulsion that forms. Allow to stand undisturbed for 10 min. If the amount of flour entrapped in the gasoline appears to be small, trap off the gasoline and filter thru a rapid action paper, using suction. Add an additional 20 ml of gasoline to the ma-

terial in the Wildman trap flask. Stir the gasoline into the soln and after several minutes trap off and filter thru a second paper. Examine the papers microscopically.

"If, originally, the amount of flour entrapped in the gasoline appears to be relatively great, proceed as follows: Trap off the gasoline in the usual manner into a 250-ml beaker. Again add 20 ml of gasoline to the material in the trap flask. Stir the gasoline into the soln and after 3 min. trap into the same beaker. Transfer the contents of the beaker to a 2-liter Wildman flask; fill with H_2O. Stir, and after about 30 min. trap off the gasoline and filter thru a rapid action paper, using suction."

"Macroscopic Examination of Rolled Oats and Similar Material"

"Weigh 225 g and sift thru No. 6 sieve using small portions of oats at a time. Remove clumped oats for further examination. Repeat the above examination on a No. 8 sieve and, if pertinent, a No. 10 sieve. Pass the siftings thru a No. 20 sieve. Transfer the material on the No. 20 sieve portion-wise to a white tray and examine for insect and rodent filth. Examine the portion passing thru the No. 20 sieve for insect excreta.

"Check clumped material microscopically for insect webbing and frass; for urine, as indicated by fluorescence under ultra-violet light and the presence of urea (**42.97, .98, .99, .100**). If insect excreta is sifted thru the No. 20 sieve, transfer it by washing with $CHCl_3$ or CCl_4 to a ruled filter paper in a Hirsch funnel. Treat and examine as in **42.31** "

STRAWBERRIES (FROZEN)

42.42 (b) Delete, line 2, "or thru No. 30 sieve, using a stiff brush."

APPLE BUTTER

42.43 Add following at end of paragraph:

"Rot fragments—See **42.66 (b)**."

42.44 Insert "mix well," after the words "castor oil."

FRESH, CANNED, AND FROZEN BLACKBERRIES, ETC.

42.47 After word "diam" 3d line from end of last paragraph strike out "or thru a No. 30 sieve using a stiff brush."

42.49 Add "Note: This method does not appear to be satisfactory for examination of fresh or frozen blackberries that have been bleached with sulphite."

Add the following method at end of paragraph:

"*Maggot recovery.*—Transfer residue from trap flask to a black-bottomed pan. Decant the H_2O and coarse pulp slowly from the pan. Add more H_2O and repeat the decantation. Pick out the maggots found by a careful examination of the contents of the pan. Transfer the contents of this pan to a white-bottomed pan and pick out and count the maggots found in this pan."

Add following:

"*Alternative method for maggots.*—See new procedure substituted for **42.50** and **42.51** below."

42.50 Delete and substitute the following procedure for **42.50** and **42.51**:

"MAGGOTS"

"In Blueberries and Cherries"

"Weight 567 g (20 oz) of fresh fruit or use a No. 2 can of processed fruit. Add 100 ml of H_2O to the fresh or frozen fruit and boil 5 min. with frequent stirring. (Omit this step if fruit is canned.) Transfer a ½" layer of fruit to a No. 6 sieve immersed in a pan of H_2O. Shake the loose maggots and debris thru the sieve. Mash the fruit

carefully under H_2O in order to rub any remaining maggots thru the sieve. Rinse and discard the pulp and seeds, if any. Repeat the above process with another portion of the fruit. After all the fruit has been screened, transfer the mixture to a black-bottomed pan. (With cherries transfer first to a No. 6 sieve resting in ca 1" of H_2O. Shake the sieve until the maggots drop thru, and discard the pulp on the sieve.) Slowly decant the H_2O and pulp from the pan. Add more H_2O and repeat the decantation. Pick out the maggots found by a careful examination of the contents of the pan. Transfer the contents of this pan to a white-bottomed pan and pick out or count the maggots found in this pan."

42.51 Delete and substitute procedure referred to in **42.50** above.

42.52 Change heading "Fig and Prune Paste" to "Fruit Paste."

Delete and substitute the following methods:

"LIGHT FILTH"

"Place 100 g paste in a 2-liter trap flask. Add ca 400 ml H_2O. Heat. Continue boiling ca 10 min., or until the material is disintegrated. Cool to 20–25°. Add 30 ml kerosene. Mix. Fill the flask with H_2O. Allow to stand ca 30 min., stirring the lower layer 5–6 times during this period. Trap off the oily layer. Add 30 ml more kerosene; re-mix, and, after stirring down the upper layer several times, again trap off. Pour the trapped-off material thru a No. 20 sieve. Rinse the sieve. Filter the material passing thru the sieve and examine. Rinse the material retained on the sieve to a beaker. Filter and examine.

"INSECT HEADS IN FIG PASTE AND FIG SLICES"

"Place 100 g paste or fig slices in a 2-liter trap flask. Add 500 ml warm (30–50°) H_2O. Boil with intermittent stirring until the paste is disintegrated and concentrated to ca 400 ml. Cool to ca 50–60°. Add 10 g NaOH. Bring to a boil and continue boiling briskly for ca 5 min. Cool to ca 50°. Add 20 ml conc HCl. Cool again to ca 50°. Add 30 ml castor oil. Stir, add H_2O until the oil comes ca half way into the neck of the flask. Let stand 15 min. Agitate and with the plunger work down the floating fruit pulp and seeds with a downward motion that breaks the interface. Repeat this twice. Add warm (ca 50°) H_2O to release those seeds at the surface of the oil. Let stand ca 30 min., stirring the lower layer ca 5–6 times during this period. Trap off, filter, and examine."

CANDY

42.54 At end of first sentence add "Examine the surface of the candy for gross contamination with the naked eye or by using a low-power magnifier. Observe and note the character and extent of the contamination. Report the number and weight of the contaminated pieces of candy."

42.54 (a) Substitute the word "boiling "for "hot" in the 1st line.

42.54 (b) In 4th line change "50°" to "40°."

42.54 (c) After "temp." 2nd line, add "or stronger up to ca 10% $Na_2B_4O_7$."

After "to boiling" 2nd line, add "or in boiling H_2O."

42.54 (d) In 6th line, after ( ), add "The use of the coarse sieve may be omitted if only a small number of fruit or nut particles are present."

42.54 (e) Add to end of paragraph:

"Extract the residue from the coarse sieve with alcohol and gasoline or kerosene as described in **(d)**."

42.54 Add the following procedure as new paragraph:

"*In candy containing corn flakes or similar cereal fillers.* Dissolve the candy by boiling in a large quantity of HCl soln (1+19) and use the appropriate method listed above for the particular type of candy."

Add the following procedure as new paragraph:

"*In chocolate candy coating for insect excreta and other filth.*—Heat 400 ml gasoline in an 800-ml beaker to 40–50° and maintain it at this temperature. Place a portion of the candy in a wire basket (ca 3¼" diameter×1" high) made from No. 8 screen and with wire handles. Move the basket up and down thru the gasoline until the chocolate coating is dissolved. Rinse each candy center with a fine stream of gasoline from a wash bottle and save the center. Repeat with the balance of the sample. Stir the gasoline-chocolate suspension and pour thru a No. 140 sieve. Transfer the residue from the sieve to a filter paper and examine microscopically. Examine the candy centers by an appropriate method."

TOMATO PRODUCTS (NOT DEHYDRATED)

42.59 Substitute following paragraph for first 13½ lines ending . . . "to make volume to 20 ml."

"Weigh the sample (paste 1 g, puree 2 g, juice 5 g) into a 50 ml graduated cylinder which has been cut off at the 30 ml mark. Make up to approximately 10 ml with H_2O, mix thoroly, add 1 ml approximately N/1 sodium hydroxide soln and 4 drops of a 10% alcoholic soln of crystal violet. Mix well and let stand 3 min. Make up to the 20 ml mark with approximately 1% algin soln and mix well."

TOMATO SOUP, CANNED SPAGHETTI, ETC.

42.64 (a) Substitute for "KOH soln (1+1)" line 3, the following:

"NaOH (1+1). If starch is absent omit NaOH."

In line 10 change "KOH" to "NaOH."

42.64 (c) Delete and substitute following procedure:

"*Tomato sauce packing medium on fish.*—Drain off the oil from the fish in the can as much as possible without disturbing the tomato sauce covering the fish, which is usually relatively free from oil or fish tissue. Use paper toweling or blotting paper to remove oil at the edges of the sauce area or from any depressions where the oil does not drain off readily. Pour ethyl ether gently from a beaker over the sauce on the fish while holding the can in an inclined position so as to allow the ether to drain. With a scalpel, sample the sauce directly for a mold count, selecting from areas of tomato sauce comparatively free from the presence of fish tissue. If a rot fragment determination is desired, remove sufficient sauce to a small beaker in which the sauce can be further cleared by swirling with small amounts of ether and decanting. The residual ether evaporates at room temperature in a short while, although the process may be hastened by cautious warming. Do not attempt to count the material if the tomato sauce is too considerably mixed with fish material. Count as directed in **42.57**."

42.71 Add as new method:

CANNED GREENS AND BROCCOLI

"Aphids in Canned Greens"

"Determine drained weight of the contents of canned greens in the usual way, reserving the drained liquor. Chop the drained leaves into pieces approximately 1–2 inches in length, weigh 100 g of the well-mixed sample into a 1-liter beaker. Add H_2O to adequately cover, followed by 25 g neutral lead acetate crystals (or equivalent solution of lead acetate) and 10 ml glacial acetic acid. Boil on a hot plate for 5–10 min., cool, and transfer to a 2-liter Wildman trap flask. Add 35 ml gasoline, mix thoroughly so as to assure contact between the gasoline and all portions of the leaves. Fill the flask with deaerated H_2O. Allow to settle a few minutes during which most of the vegetable matter sinks to the bottom. Usually some tissue rises, prob-

ably held by entrapped globules of gasoline. To force this material to sink, pivot the lower end of the rod of the Wildman trap on the bottom of the flask, and rotate the upper part of the rod around the neck of the flask with a view to knocking the globules from vegetable tissue without at the same time breaking the interface and thus re-wetting the tissue with gasoline. Again allow to stand, trap off the gasoline layer and filter. Re-extract with 20 ml of gasoline, trap off and filter (usually possible on the same paper). Determine the total aphids or other light filth in the entire liquor drained from the can by subjecting it to gasoline flotation in the usual manner. (The liquor does not normally present any difficulty and the use of lead acetate is unnecessary.)

"Count only whole aphids and thrips. They may be mutilated, crushed, or up to one-half of the insect may be missing, and yet counted if the head is present, but fragments less than one-half and cast skins are not to be included in the count. Cast skins are recognizable from their pale ghostlike appearance and complete absence of interior. Count fragments of insects other than aphids and thrips and other evidence of filth separately. Calculate on basis of weight of drained material."

MUSHROOMS

42.75 Strike out period, line 3, after "overnight." and add:

"on a steam bath, or boil for 30 min. Cool to room temperature."

Strike out words "after soaking," line 3, and capitalize word "Add."

Strike out last sentence and substitute:

"Filter through 10XX cloth and examine."

SWEET CORN

42.77 Delete first paragraph and substitute:

"Use a No. 2 can or equivalent as sample."

Add at end of method:

"Empty the residue of corn remaining in the bottom of the flask when trapping is complete onto a five-inch No. 10 sieve. Place on the sieve the remainder of the corn from the No. 2 can or equivalent and wash under the tap to remove starch and fine particles. Place residue on the sieve in a pan and examine under H_2O for worm-eaten or rotten kernels."

GROUND SPICES

42.78 Insert underneath "Ground Spices" the following paragraph:

"*Gross Contamination.* Sift 200 to 400 g of the ground spice thru a No. 20 sieve. Transfer any insects or other filth retained on the sieve to a suitable dish and examine with a Greenough microscope. Report the filth on a 50 g basis.

Change the title "Allspice, Ginger, etc." to read "*Ground allspice, anise, caraway, cardamom, celery seed, cloves, coriander, cumin, curry powder, dill seed, fennel, fenugreek, ginger, mace, marjoram, mustard, nutmeg, oregano, poppy seed, rosemary, sage, savory, and thyme.*

In 4th line beginning "add 150 ml of CCl_4," substitute "$CHCl_3$" for "CCl_4."

In 5th line substitute "$CHCl_3$" for "CCL_4."

In 6th line strike out "Add more solvent and repeat if necessary" and insert following sentence: "If appreciable spice tissue remains in bottom of beaker, add successive portions of $CHCl_3$ mixed with CCl_4 to give increasingly higher specific gravities until practically all spice tissue is floated off."

In 11th line change "5" to "15." After word "stirring" add "wash down inside of flask with H_2O and cool to below 20°." Strike out "then cool to room temp."

Insert following after **42.78**:

"*Whole, cracked, or pieces of allspice, anise, bay leaves, caraway, celery seed, cin-*

namon, cloves, coriander, cumin, dill seed, fennel seed, fenugreek, ginger, mace, mixed pickling spice, mustard, nutmeg, oregano, black pepper, white pepper, poppy seed, rosemary, sage, and turmeric."

"Filth. Weigh 25 g samples and follow procedure in **42.78** using a larger beaker and more reagent where necessary."

42.79 Change title to "Ground cinnamon."

42.80 Add the title "Ground cinnamon and turmeric."

42.81 Delete.

42.82 Add following as 2d paragraph:

"Draw air thru the plant tissue in the Büchner funnel until the solvent is evaporated. Transfer to a 1-liter Wildman trap flask **42.1(a)**. Add 200 ml of hot H_2O and 20 ml of castor oil. Mix thoroly and fill the flask with hot H_2O. Allow to stand 30 min., stirring every 5 min. Trap off the oil layer and filter. Add sufficient hot H_2O to fill the trap flask and trap off a second time after 15 min."

42.83 Change title to read "Ground Black and White Pepper."

42.84 Insert "Ground" before "Capsicums" in title.

Line 14 strike out "saturated" and insert after "soln," "saturated at room temperature."

After paragraph **42.84** insert the following method:

"Whole Marjoram, Savory, and Thyme"

"Filth. Weight 25 g sample into 400-ml beaker and follow procedure in **42.78** 'Light and heavy filth' with the following modification: Use more reagent where necessary. Use a 2-liter Wildman trap flask and add 400 ml hot H_2O+20 ml HCl. Use 35 ml gasoline instead of 25 ml."

MAYONNAISE AND SALAD DRESSINGS

42.90 Insert the word "mayonnaise" after the phrase "Again mix thoroly and filter." Insert "and salad dressing thru a 10XX bolting cloth with suction" after the words "filter thru rapid paper." Remove words "with suction" following "thru rapid paper."

PICKLES AND RELISHES

Transpose "trapping off" and "allowing to settle 10–15 min.," line 13.

42.91 Add at end of paragraph: "If only a small amount of debris is being washed from the pickles it may be washed directly onto the filter paper. State which procedure is used."

42.92 Insert after the words "H_2O to 100 g of pickle," "in a trap flask or beaker." Insert after the words "boil 15 min., and cool," "If boiling is not done in a trap flask, place in a" . . . etc.

CONDIMENTAL SEEDS

42.93 Insert as 2d paragraph the following:

"Alternative Procedure.—Weigh 200 g of the seeds into a suitable container and examine carefully with the aid of a jeweler's loupe or other magnifier of ca 6X power. Separate the rodent, insect, and other excreta and weigh each to the nearest mg."

42.95 Delete "WHOLE SPICES" and paragraph.

CLOTH

42.97 Add following sentence:

"When odor of urine is detected, report this finding."

42.98 In the last part of paragraph remove the lines beginning with: "(When presence of urine stains cannot" . . . etc. to the end of the parenthesis. At the close of the

paragraph add the word "urea," after "based on the formation of" and before "urea nitrate."

Add following as 2d paragraph:

"Stained patches of the food material can be tested by a procedure similar to the above."

(2) The following additional methods were tentatively adopted:

Canned Fish

Filth.—If the portion consists of 1 pound or less transfer the total contents of the can to a 2-liter trap flask. Reduce larger portions to 1 pound. Avoid breaking the fish into small particles. Cover fish with hot water; rinse the can and lid with 25 ml kerosene allowing the rinsings to enter the trap flask. Mix. Fill with warm water. Trap off, filter, and examine. Trap off a second time, using 20 ml kerosene, filter and examine.

Chicken Giblet Paste

Filth and sand.—Weigh 100 g into a 400-ml lipped beaker. Add $CHCl_3$ to within one inch of the top. Mix thoroly and allow to settle for ca 30 min. stirring the top layer several times during this period. Make an opening in the floating layer and decant off most of the $CHCl_3$. Add, stir, and decant off the $CHCl_3$ three times. Retain the decanted $CHCl_3$ and filter thru a 100-mesh bolting cloth. Examine microscopically. After the third extraction decant the paste layer into a 2-liter Wildman trap flask. Evaporate most of the $CHCl_3$ from the material in the flask.

Add ca 50 ml hot H_2O to the residue in the beaker. Decant and wash the residue thru an ashless filter in a 60° funnel. Wash with warm H_2O. Remove the filter to a Petri dish and examine it for rodent excreta, sand, etc. To weigh sand, etc., fold the filter paper, dry in a tared crucible, ignite, and weigh to the nearest 0.5 of a mgm.

To the paste in the trap flask, add 200 ml hot H_2O and heat on a steam bath for 15 min. Cool to room temperature. Add 35 ml gasoline, mix, allow to stand 10 min. stirring several times. Trap off with H_2O, filter, and examine.

Meat Scraps

Glass.—Weigh 50 g of well-mixed sample into a 400 ml beaker. Add ca 350 ml CCl_4 and mix contents thoroly. Allow to stand with occasional stirring for 30 min. Decant and discard the CCl_4 and floating organic matter, leaving bone material and heavy matter behind. Add more solvent and repeat if necessary. Wash out adhering CCl_4 with one rinse of ca 100 ml alcohol and then one rinse of 350 ml water, decanting slowly after each alcohol or water addition so that no heavy matter is lost. Add 50 ml of conc HCl and heat on a steam bath for ca one hour. Wash the residue with 3–6 portions of water, 350 ml each rinse; then with one rinse of ca 50 ml alcohol and then 2–3 rinses of CCl_4; decant after each addition and finish with a rinse of chloroform. Allow the heavy residue to dry thoroly and weigh to the nearest mgm. Pass the dry residue thru No. 40 and No. 60 sieves and weigh the total residue on each sieve and the material passing thru the No. 60 sieve. With the aid of a Greenough-type microscope pick out and weigh the glass from the fraction retained on the No. 40 sieve.* Estimate the per cent of glass in each portion passing thru the No. 40 sieve.**

* All particles of glass should be checked with the polarizing microscope since particles of clear quartz may often be mistaken for glass unless examined with polarized light. Since glass is isotropic in character it will show complete extinction of the transmitted light when examined between crossed nicols, while quartz which is double refracting will exhibit polarization colors.

** The estimate may be accurately made by determining the percentage of glass particles from a microscopic examination of a minimum of 200 random particles from each portion, using polarized light.

Chewing Gum

Filth.—Add 100 g of gum to ca 300–600 ml H_2O or ca 150–600 ml of 2% HCl soln. Bring to a boil and continue boiling for ca 10–12 min. To prevent excessive caramelization do not boil any longer than is necessary to obtain a finely divided mixture of the gum particles in the liquid.

At this point the dispersed gum can be treated by either of the following procedures to dissolve or soften the chicle:

(1) *Optional.*—Allow to cool to ca 55°. Add 150 ml acetone; stir and then add 150 ml chloroform. Bring to a boil and continue boiling until the mixture is dispersed evenly. While hot, pour thru a 10XX bolting cloth using suction in a Hirsch funnel.

(2) *Optional.*—Cool to either ca 80° (if carbon tetrachloride is to be used) or ca 63° (if chloroform is to be used); add ca 150 ml carbon tetrachloride or chloroform and simmer for 5–10 min. or until the chicle has dissolved. While hot filter thru a 10XX bolting cloth using suction in a Hirsch funnel.

CAUTION: The mixtures have a tendency to foam and boil over. Take adequate precautions against fire or hazardous prolonged breathing of the vapors from the organic solvents. All mixtures should be stirred while adding liquids and while being heated to boiling. When transferring to a new container, take care to rinse the old container with hot H_2O and the appropriate organic solvent. The filtering funnel may be greased to prevent the cooling gum from sticking to it.

Technical grade chloroform and carbon tetrachloride may be used.

Shredded Coconut

Filth.—Weigh 100 g in an 800 ml beaker, add 500 ml of hot 1% sodium oleate soln, and bring to boiling. Decant the soln into a 2 liter Wildman trap flask thru a No. 6 or 8 sieve of suitable mesh. Place a thin layer of the coconut on the screen and wash with fine strong stream of H_2O, successively removing the layer and adding more until the entire sample has been rinsed. Discard washed coconut. Catch all washings in trap flask with the oleate soln. Cool to room temp., add 30 ml of gasoline, and mix thoroly. Fill the flask with H_2O and allow to stand 1 hour, stirring occasionally. Trap off and filter thru 10XX bolting cloth and examine.

Coarse Peanut Butter

Rocks and decomposed peanuts.—Remove the entire contents of the jar to a 1500-ml beaker or other suitable container. Add ca 700 ml CCl_4 and mix thoroly, using, if convenient, the electric mixer described in the *Methods of Analysis*, 6th ed., **22.109**. Rinse the jar with CCl_4 and add this to the beaker. Allow the mixture to stand at least 15 min. with occasional stirring. Decant ca $\frac{2}{3}$ of the mixture and add ca 200 ml CCl_4. Allow to stand 5 min. and decant. Wash down the sides of the beaker with CCl_4 and repeat the decant until the residue is free from peanut tissue. Save all decanted material. Dry the residue in the beaker and wash out the salt with hot H_2O. If considerable sand is present test the residue for salt, phosphate, carbonate, and anhydrite as directed in **42.24**, second paragraph. Transfer the residue to an ashless filter paper and examine under the low-power microscope. Report the number and approximate size of the rocks and other extraneous material. If considerable sand is present, ignite the filter and weigh the residue, including the rocks, reporting the result in mgm per 100 g of peanut butter. State the number of jars and weight of material examined.

Pour the decanted CCl_4 and peanut mixture thru a No. 14 sleve and examine the residue for gross filth, stems, other extraneous material, and decomposed peanut tissue. Report the kind, amount, size, and weight of such material.

Jams and Jellies

INSECT AND RODENT FILTH

(a) *Jam.*—Empty the contents of the jar into a dish and mix thoroly. Weigh 100 g into a beaker, add 200 ml of H_2O (ca 50°), transfer to a 1-liter Wildman trap flask, add 10 ml HCl, and boil ca 5 min. Cool to room temperature. Add 25 ml of gasoline and stir thoroly. Trap off, filter, and examine.

(b) *Jelly.*—Empty the contents of the jar into a dish and mix thoroly. Weigh 100 g into a beaker. Add ca 300–400 ml of hot H_2O. Warm the beaker, with stirring, until the jelly goes into solution. When dissolved, filter. Examine for filth.

Occasionally so-called jellies will not filter thru paper because of the presence of small amounts of fruit tissue, in such case use (a) above.

Apple Pomace from the Manufacture of Apple Cider

ROT AND WORM FRAGMENTS

(a) *Kerosene flotation for insects and light filth.*—Place 200 g of wet pomace in a 1500 ml beaker, add 90 ml of H_2O and boil for one min. Transfer while hot to a 2-liter Wildman trap flask. Add 35 ml of kerosene, trap off, and examine as in the usual flotation method. Stir and make a second trapping of the kerosene-water layer after it has stood for 15 min.

(b) *Bleaching with sulfur dioxide for rot fragments and worm injury.*—Place 200 g of the well-mixed sample of wet pomace in a quart glass-top Mason jar. Fill the jar ca ⅔ full of H_2O. Add ca 10 g of anhydrous sodium sulfite and 10 ml of conc. HCl. If not sufficiently decolorized, decant the supernatant soln and repeat the process.

Drain on a No. 30 sieve and wash with cold running H_2O until there is little odor of SO_2. Drain until there is practically no H_2O dripping from the pomace. Weigh 100 g of the well-mixed sample. Remove a small quantity at a time to a Petri dish and examine with a Greenough-type microscope at 10 diam. Pick out the wormy and also the decomposed fragments (not wormy). Repeat until the entire sample is examined. Use care not to confuse bruised tissue with decomposed tissue. Examine the decomposed tissue for mold filaments with the compound microscope if in doubt. Weigh the insect-infested fragments and the decomposed fragments separately. Count and report any insect excreta pellets found. Report the addition of insect-infested fragments and decomposed fragments as percentage by weight.

Frozen and Canned Blueberries

ROT

(a) *Macroscopic examination.*—In the case of frozen blueberries allow each subdivision to thaw at room temperature. Prepare stock bleaching soln by adding 1 teaspoon of $NaHSO_3$ to a qt. of H_2O. Spread the thawed-out berries in a shallow pan. Remove 100 berries at random and place them in a small bottle or suitable container and cover with bleaching soln. Let stand an hour or two or until the berries are bleached, drain off the soln, and place in a white-bottom pan and cover with H_2O. Separate berries into those showing rot and good berries. Confirm all questionable rots by examining fragment of tissue under compound microscope. Classify as rotten all berries which contain a large number of solid filaments. Determine the number of rotten berries and report as per cent of rotten berries. Preserve all of the separations in small bottles adding some of the bleaching soln as preservative.

(b) *Microscopic examination.*—Pulp the remainder of the sample thru cyclone with screen opening .027″ in diam. and discard residue. Weigh out 50 g of the well-mixed sample and add 50 g of 3% pectin soln **42.2(b)**. Stir thoroly and make a mold count as directed under **42.57**. In the event that seeds are taken up with the drop to the mold-counting disk, they should be removed by means of a pair of fine-pointed forceps.

Raisins and Currants

INSECT INFESTATION, MOLD, AND SAND OR SOIL

(a) *Insect infestation.*—Count out 100 berries. Place them in a large white enameled pan and wet gently with a fine stream of alcohol. Add sufficient H_2O to cover the fruit. The fruit may be placed in the depressions of a white-spot plate or in muffin pans and similarly treated. In the case of the spot plate or muffin pan the use of one berry in each depression may obviate the confusion that might arise thru mixing residues from adjacent berries if the larger pan is used. When the large pan is used, arrange the berries in rows so that the berries are at least one inch apart. Soak the fruit ca 30 min. Examine each berry using a binocular loupe or a watchmaker's glass with a magnification of ca 5 diam. Tap or knead each berry with a pair of laboratory forceps to release the excreta pellets.* (see note)

(b) *Mold.*—Empty the container and mix well. Count out 100 berries. Place them in a 400 ml beaker, cover with H_2O, heat to boiling and boil ca 5 min. Drain off H_2O and place raisins in a white-bottomed pan. Cover completely with H_2O. Examine with an ocular loupe or jeweler's eye piece with magnification ca 5 diam. Pick out those berries which are obviously moldy—those that are a light grey color and are soft and mushy—and those that are suspected of being moldy. Examine these microscopically to determine the presence of mold filaments. Count and report number and per cent total moldy berries present.

(1) *Alternative.*—In lieu of boiling 5 min. the 100 berries may be placed in a pan as above and allowed to soak either overnight or from 30–45 min. and then examined as above.

(c) *Sand or soil.*—Mix sample and weigh out 100 g. Boil in H_2O ca 5 min. Rinse the raisins on a No. 10 sieve, using a fine stream of H_2O, catching the rinse H_2O. Allow to settle 10–15 min. Decant off the upper portion of liquid and then filter thru an ashless filter. Ignite to an ash, weigh, and determine amount present. Report as mg per 100 g.

Lingon Berries

CATERPILLARS

Procedure.—Place 1 pound of the berries and juice in a No. 10 can or other convenient container. Fill with H_2O to within ca $\frac{3}{4}$ inch of the top. If frozen, permit berries to thaw. Stir several times and pick out larvae which float to the surface. Pour the mixture into a No. 6 sieve, passing the liquid thru a No. 40 sieve. Rinse the berries on the No. 6 sieve with H_2O and pass this rinse water thru the No. 40 sieve. Rinse the material on the No. 40 sieve into a white pan and pick out the insects.

Boil the berries from the No. 6 sieve for 15–20 min. Cool. Mash thru a No. 6 sieve. Mix the mashed material with saturated salt soln in large container (*e.g.*, No. 10 can). Stir several times and pick out the insects.

Currant Pulp

INSECT AND RODENT CONTAMINATION

Procedure.—Weigh out a 50 g portion of the sample and boil it in ca 400 ml of H_2O. Place a No. 14 screen over a 1-liter beaker and pour the boiled sample into the beaker thru the screen. Rinse out the beaker in which the pulp was boiled with additional H_2O and pour the rinse water over the screen. Wash the material left on the screen into a large evaporating dish and add this wash water to the filtrate in the beaker. Transfer the filtrate from the beaker to a 1-liter Wildman trap flask

* In reporting "insect infested," report pellet counts in ranges. Thus, those having from 1–5 pellets are reported in one group; those having 6–10 are reported in a second group; those having 11–15 are reported in a third group, etc.

and add 35 ml of gasoline. Mix thoroly by vigorous stirring. Fill the flask with H_2O to bring the gasoline layer up into the neck of the flask. Stir vigorously and allow to stand 15 min. Trap off and pour the trapped portion into a beaker. Add more H_2O to the flask, stir, and allow to stand 10 min. Again trap off and pour this portion into the beaker. Filter, using a Hirsch or Büchner funnel. Several papers may be necessary and care should be taken to filter slowly so as not to accumulate too much pulp material on the paper. Examine the filter papers.

Grape Pulp

ROT

Thoroly mix the pulp. Weigh out a 100 g portion and rinse into a Waring Blendor with 100 ml of 0.5% NaOH soln and mix for 3 min. Break any resulting foam by adding 2–3 drops of caprylic alcohol and stirring. To 20 g of this blended pulp add 20 g of a 4% pectin soln. Stir thoroly and make the usual Howard mold count.

Dried and Canned Fruits (apples, peaches, pears, apricots, plums, and prunes)

FILTH

(a) *Canned.*—Drain off liquid. Rinse the fruit with H_2O. Filter and examine packing medium and rinse water. Include amount examined, gross and rinsed weight, number of pieces of fruit, and condition of fruit (whole, halves, pitted, peeled).

(b) *Dried.*—Count out 100 pieces (or if in pound or half-pound boxes use entire contents of box). Soak for a few min., or for just long enough to loosen any adhering contamination. Rinse each piece with H_2O. Filter soak and rinse water and examine.

Dried Mushrooms

Maggots. Rapid test for gross examination.—Place 100 g in a beaker and cover with hot H_2O. Boil 30 min. Pour onto a No. 8 sieve in a suitable pan. Add sufficient H_2O to cover the mushrooms. Release the filth by rubbing the mushrooms on the sieve. Filter thru a 10XX bolting cloth. Repeat the extraction process as many times as necessary to recover all the heavy filth.

43. STANDARD SOLUTIONS

The following method for standardization of thiocyanate solution was adopted as official, first action.

REAGENTS

(a) *Purified silver nitrate.*—Dissolve 50 g reagent $AgNO_3$ in 20 ml of boiling H_2O containing ca 5 drops HNO_3. Heat to effect soln and filter while still hot thru a fritted glass filter, using suction, and collect filtrate in a clean Pyrex beaker. Wash beaker and filter with about 5 ml hot H_2O, adding washings to filtrate. Cool in an ice bath, stirring to induce crystallization, and place in refrigerator at about 10°C. until equilibrium is reached. Decant liquid thru fritted glass filter and transfer the crystals to filter. Cover filter with a watch-glass and draw air thru filter to remove adhering liquid. Transfer crystals to small, clean Pyrex beaker. Cover beaker with watch-glass and place it inside a larger covered Pyrex beaker. Dry at 105°C. and fuse at 220–250°C. (melting point 208°C) holding at this temperature about 15 min. after crystals are melted. (4) Protect from entrance of dust during preparation. Cool in desiccator, remove product from beaker, powder in mortar, dry ½ hour at 105°C., and preserve in brown glass-stoppered bottle in dark place over good desiccant.

(b) *Reference soln.*—To 5 ml HNO_3 (**43.26(b)**), 2 ml ferric-alum soln (**43.26(a)**),

115 ml H_2O, add about 0.02 ml 0.1*N* thiocyanate soln, noting the exact quantity used.

STANDARDIZATION

Weigh accurately on tared watch-glass sufficient quantity of $AgNO_3$ reagent (a) to give titration of approximately 40 ml (about 0.7 g in the case of 0.1*N* soln) and transfer with water thru glass funnel to 250 ml glass-stoppered Erlenmeyer flask. Dissolve in about 75 ml H_2O (halogen-free) and add 5 ml HNO_3 **(43.26(b))** and 2 ml of ferric indicator **43.26(a).** Titrate with thiocyanate soln to appearance of reddish-brown color which remains after shaking vigorously for 1 min. Record buret reading and set flask aside for 5 min., shaking occasionally. Maintain end point color during this period by addition of thiocyanate soln as required. At the end of 5 min. period add additional thiocyanate soln, if necessary, to produce permanent end point color, matching with color of reference soln **(b)**. From total volume of thiocyanate soln used in titration subtract the quantity contained in reference soln. Calculate normality of soln using the following formula:

$$N = \frac{\text{gm } AgNO_3}{\text{ml titer} \times 169.89/1000}$$

ERRATA AND EMENDATIONS, METHODS OF ANALYSIS, A.O.A.C., 1945

The errors that are reported from time to time by those using this book will be published in *This Journal.* The following changes should be noted.

Section	*Page*		
2.12	23	line 8, par. (a)	Insert the word "paper" between the words "litmus" and "with."
2.51	35	line 1, par. 2	Delete "If alcoholic filtrate is clear, the $Ca_3(PO_4)_2$ will not exceed 0.3 mg and may be neglected."
11	115	Ref. (5)	Change "Ridgeway" to "Ridgway."
12.47	132	line 26	Change "Na_2SO_3" to "$Na_2S_2O_3$."
12.53 (b) (1)	134	line 5	Change **"34.38"** to **"34.39."**
12.54	134	Title	Change **"27.22"** to **"27.24."**
12.55	134	Title	Change **"27.27"** to **"27.28."**
12.59	135	line 10	Change **"2.19** to **"2.22."**
12.75 (a)	144	line 2	Change **"12.69"** to **"12.70."**
13.23	149		Delete "(*See* **16.68**)" and insert "Proceed as directed in **16.68** collecting about 300 ml of distillate."
14.2	151		Change **"14.55"** to **"14.60."**
14.44	160	line 2	Delete period after **"14.43"** and add "Cut the kernels in longitudinal halves."
14.57	165	line 11	To"Plato"in parenthesis, add"**,44.3.**"
14.57	165	line 13	Change "5 g" in numerator of formula to "50 g"
14.58	165	line 3	Change **"14.55"** to **"14.60."**
14.63	167	line 1, par. 3	Between "power" and "of" add "with aid."
14.69	169		Change **"14.56"** to **"14.50."**

Section	*Page*		
14.75	170	line 2	After "Plato's" table, add ",44.3."
14.104	176		Change title to "Original Extract or Original Gravity."
14.119 (c)	180		Substitute "14.74" for "14.56 (c)" and add "Proceed as for flaked corn and flaked rice."
14.121	180	last line	To "Plato" in parenthesis add ",44.3."
14.122	181	line 2	After "14.121" add "Make mash to 450 g."
14.122	181		Delete "where" after 1st formula and insert it after 2d.
14.122	181	last line	To "Plato" in parenthesis add ",44.3."
14.124	181	line 2	Delete "where." Add "% soluble extract, dry basis $=\frac{E\times100}{100-M}$, where"
		last line	After the comma add "E = soluble extract 'as is'." To "Plato" in parenthesis add ",44.3."
14.92	174	line 13	Place the denominator "P" beneath "(p − n) × 100."
16.8	193		Change "Tentative" to "Official" in title.
16.11	194		Change "Tentative" to "Official" in title.
16.13	194		Change "Tentative" to "Official" in title.
16.22	196		Change "Tentative" to "Official" in title.
16.25	196		Change "Tentative" to "Official" in title of "Modified Denigès Method."
18.26	219	line 1	Change "H O" to "H_2O."
19.15 (a)	226	line 2	Change "nursing" to "centrifuge."
19.15 (b)	226	line 2	Change "nursing" to "centrifuge."
19.16 (a)	227	par. 3	Delete the sentence beginning line 5 and ending line 6, reading "Dilute to ca 90 ml and precipitate pectic acid with 10 ml of 15% NaOH soln."
19.16 (b)	228	line 7	After "(a)," insert "(line 5)."
19.42	234	line 1	Change "8 oz. nursing" to "250 ml centrifuge."
20.8	238		Change designation "Tentative" to "Official, first action."
20.28	245	line 5	Change "30" to "10."
20.96	262		Change "Official, first action" to "Official."
22.137	340	line 12	Delete "50 ml of."
22.143	342		Add "(38)" after word "cream" in title.
22.146	343		Add "(39)" after word "sample" in title.
22.147	343		Add "(40)" after word "solids" in title.

Section	*Page*		
22.147	344	Ref. (37)	Add "23:597 (1940), 28:245 (1945)."
	344	To section on selected references, add the following:	"(38) *Ibid.* 28:601 (1945) (39) *Ibid.* 25:614 (1942) (40) *Ibid.* 24:575 (1941)."
23.3	346	line 6	Delete "weight of egg residue as."
23.26	353	line 4	Change "an hydrous" to "anhydrous."
	358	Ref. (12)	Change "22" to "22."
23.36	357	last line	Change "Filter-Eel" to "Filter-Cel."
25.1	365		Change "14.3" to "16.4."
25.25	369		Change "16.26" to "16.28, 16.29, or 16.34."
27.59	419	line 10	Insert "15" between "each" and "mg."
29.13	441	line 15	Last word is "present."
29.13	441	line 17	Change "deis" to "dis-"
29.26	447	line 1	Letter between "furnish" and "representative" is "a."
32.30	533		Insert cross reference under title "Quantitative, see 29.22–29.33, incl.
33.36	545	line 3	After "HNO_3" add "Bring to boiling on a hot plate. Add to the boiling mixture 15 ml of 5% $KMnO_4$."
34.23 (b)	565	last line	After "44.3" add "col. 3."
34.45	574	par. 4	Add sentence "Obtain weight of reducing sugars equivalent to weight of Cu from 44.12."
34.46	575	last line	Change "44.12" to "44.13."
34.143	591		After "60°F." in parenthetical heading of table, add "+1.00 Be."
35.9	596	lines 2 and 3	Change "tomato" to "insoluble."
36.1	599		Add "Tentative" to title.
36.6	601	line 30	Change "234" to "201" in 6th column.
36.8	603	last line	Make "36.11, 36.13," bold face figures.
36.25	610	line 6	Change "on" to "in."
36.27 (b)	611	line 10	Transpose "on 3 successive days" to precede the words "or 15 min."
36.34 (c)	615	line 6	Change first "mg" to "ml."
39.108	700	line 3	Change "50 ml of 0.1 *N*" to "50 ml of 0.1 *N* I.
40.11 (d)	753	line 4, par. 2	Change "Head" to "Heat."
43.11	806	line 1	Change "$Na_2B_4O_7 \cdot H_2O$" to "$Na_2B_4O_7$-$10H_2O$."
43.27	809	line 11	Change "74.55" to "74.553."
44.4	821		Change "saccharimeters" in title to "saccharometers."

NOTE.—The report on "Changes in Methods" should also be consulted.

REPORT OF THE REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE OF THE NATIONAL RESEARCH COUNCIL

The undersigned designates attended the annual meeting of the Board of Governors in 1945 and one of us participated in the annual session in September 1946. Eight of the nine members of the Board are representatives of Experiment Stations and the ninth member is the Plant Pathologist of Boyce Thompson Institute.

The Institute has continued its effective efforts in exploratory work in fundamental investigation of insecticidal and fungicidal materials and in allocation of the demonstrations as to the practical value and utility of laboratory-proved materials. Some forty (40) collaborative and cooperative studies are now in active status and the support accorded these manifests the confidence accorded the Institute by industrial organizations.

It would be difficult to improve upon the evaluation embodied in the following excerpts from a letter signed by Mr. Howard P. Barss, Experiment Station Administrator of the Office of Experiment Stations, United States Department of Agriculture, and incorporated here, without his knowledge. The objective of the letter was to urge that an historical sketch be prepared by the only, and present, Chairman of the Board. The Board directed such action.

> "More than 25 years have passed since the Crop Protection Institute was founded. Its accomplishments under your leadership have contributed in a very important way to the remarkable progress made by the United States in the development of new and superior chemicals for the protection of crops against the attack of insects and diseases. In fact, had it not been for the Crop Protection Institute, it is quite probable that the United States would not now be leading the world in a new era that is utilizing more and more the amazing potentialities of synthetic organic chemistry for more effective, more specific, more economical, and safer crop pest control.
>
> "I believe that this quarter century of achievement should not be allowed to recede into the past without having the achievements of the Crop Protection Institute during this period placed on record in a simple, factual report to the National Research Council which sponsored its creation and to the scientists and the industries that joined forces to make possible the services that it has rendered to the country.
>
> "I am very serious in thinking that the history of the organization ought to be placed on record. The Institute has played an important role in pioneering the way for a new type of collaboration between scientific institutions and industrial organizations which has had most significant consequences. Some way should be found to recapitulate its accomplishments and thus prove the far-sightedness that first saw the needs and envisioned an institute, resting on a basis of voluntary cooperation, that would effectively help to meet these needs. This register of accomplishments would also provide a clear demonstration of the energy, ability, and effectiveness of the Chairman who has from the start been the mainspring and executive director of the whole unique enterprise."

The Institute has engaged an Associate Director, Dr. M. D. Farrar, and its future is full of promise.

H. J. PATTERSON
W. H. MACINTIRE

REPORT OF THE SECRETARY-TREASURER

By HENRY A. LEPPER

The annual meeting of the Executive Committee was held on Sunday, October 13, at 3 P.M. at the Cosmos Club, Washington, D. C. All members of the Committee attended. The audit of the firm of Snyder, Farr, & Company was presented, and accepted.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, INC.

BALANCE SHEET AS AT SEPTEMBER 30, 1946

ASSETS

Current assets:			
Cash, Lincoln National Bank		$22,142.52	
Cash on hand		662.40	
Office cash fund		27.63	$22,832.55
Accounts receivable		$ 8,360.21	
Less reserve for doubtful accounts		163.90	8,196.31
Inventories			8,534.73
Total current assets			$39,563.59
Investments			21,794.00
Total assets			$61,357.59

LIABILITIES AND SURPLUS

Current liabilities:			
Federal withholding tax collections			$ 13.06
Surplus:			
Balance, October 1, 1944		$45,589.81	
Add:			
Excess of income over expenses, per Exhibit C		15,115.71	
Adjustment of reserve for doubtful accounts		808.21	
		$61,513.73	
Less:			
Office furniture fully depreciated	$97.26		
Shrinkage, inventory of Wiley's Principles	48.93		
Uncollectible bad checks written off	23.01	169.20	
Balance, September 30, 1946			61,344.53
Total liabilities and surplus			$61,357.59

STATEMENT OF INCOME AND EXPENSE FOR THE TWO YEARS ENDED SEPTEMBER 30, 1946

Income		
Sales		
Advertisements		$ 1,109.70
Methods of Analysis		29,517.82
Journals		16,657.86
Total sales		$47,285.38
Less discounts, refunds, and allowances		3,779.25
Net sales		$43,505.13
Cost of sales:		
Printing and binding		19,435.28
Gross profit on sales		$24,070.85
Administrative expense:		
Salaries	$ 8,371.03	
Postage, express, etc.	1,069.65	
Stationery and office supplies	318.02	
Association and meeting expense	634.28	
Audit fees	300.00	
Over and short	9.09	
Bank and exchange charges	35.33	
Total expenses		10,737.40
Net profits from operations		$13,333.45
Other income:		
Social security refunds	$ 250.88	
Interest on investments	1,531.38	1,782.26
Excess of income over expenses, to surplus		$15,115.71

Following the decisions previously reported, *A.O.A.C. Journal.* Vol. **28,** 92, that the Association was exempt from payment of Federal Income Tax and D. C. Unemployment Compensation Tax, it was discovered that the language of the Federal Security law was identical with that of these other tax laws respecting scientific organizations operated without profit. Accordingly, a request for refund of taxes paid was made. It was allowed by the Social Security Administration with a return of $250.88.

The report of the auditor shows the *Journal* of the Association to be meeting expenses. However, there recently has been added to the cost of publishing the *Journal,* the salary of the Assistant Editor. In anticipation of additional increased costs of paper and printing, consideration was given by the Executive Committee to means of meeting such an emergency and keeping the *Journal* self-supporting. Inasmuch as the *Journal*

is dedicated to the dissemmination of scientific knowledge, it should be priced at the lowest possible figure to assure the greatest widespread distribution. Accordingly, the Executive Committee recommends that the subscription price not be increased.

In recognition of the practices of practically all other publishers of scientific journals, the Executive Committee recommends that free reprints of articles appearing in the *Journal* be discontinued. Reprints will be provided at cost, and the Secretary was instructed to establish a basis of pricing reprints.

During the past year, some discussion has come up over the designation of the term "tentative" now applied to some methods. It is recommended by the Executive Committee that a committee of three be appointed by the President to study the types of methods required by the work of the Association and the designations that would most appropriately classify and name them. The Constitution provides for associate membership. Since our last meeting, the Secretary has needed a clarification of the status of such members, and the Executive Committee recommends a committee of three be appointed by the President to study the question of membership classification and eligibility. These committees are to report at the next meeting of the Association.

Approved.

REPORT OF COMMITTEE ON NECROLOGY

Thirteen of our members have passed away since our last meeting or their recent deaths have not been previously reported. These are grouped according to the organization with which they were affiliated at the time of their death.

United States Department of Agriculture

D. F. J. LYNCH

Born in 1891 in Whitinsville, Massachusetts, he died on October 15, 1945. He received his B.S. degree in Chemistry from Harvard University in 1915. In 1923 he received his L.L.B. and the L.L.M. degree in 1924 from Georgetown University, D. C. Mr. Lynch had varied experience in chemical work. He was a teacher at different colleges, and an employee of commercial laboratories as well as government laboratories. The development of the method for commercial production of cellulose from sugarcane bagasse was one of Mr. Lynch's valuable contributions. At the time of his death, he was Director of the U.S.D.A. Southern Regional Research Laboratory in New Orleans, Louisiana. He was a member of the American Chemical Society, the American Institute of Chemists, the Cosmos Club of Washington, D. C., and a number of fraternal organizations.

WILLIAM ABBEY TURNER

Born on May 13, 1887, he died on May 20, 1946. Dr. Turner received all of his scholastic training at Yale, receiving his Ph.D. degree in 1916. He was associated with the following institutions: Massachusetts College, Yale, Pennsylvania, Wesleyan, and St. Lawrence, before coming to the Bureau of Dairy Industry in 1921. His chief interest here was in animal nutrition and mineral metabolism.

Food and Drug Administration

ALBERT C. HUNTER

Born February 23, 1893, at East Providence, Rhode Island. He died on April 13, 1946. Dr. Hunter received his B.S. degree at Rhode Island State College in 1915, M.S. degree in 1917, and Ph.D. in 1918 from Brown University. During his graduate work in Bacteriology he worked for the Narragansett Bay Oyster Company. He came to the Bureau of Chemistry as a Junior Bacteriologist in 1918. He soon became an expert in fishery products. When the Food and Drug Administration was organized in 1927, he was elected Head of the Bacteriology Unit of the Food Control Laboratory. In 1939 he became Chief of the Bacteriological Division of that laboratory. At the time of his death, he was Chief of the new Microbiological Division formed by the consolidation of the Bacteriological and Microanalytical Divisions. He was respected for his scientific ability and for his sound judgment, for his abundant common sense, and his delightful sense of humor.

HERBERT ORION CALVERY

Dr. Calvery was born in Eddy, Texas on December 9, 1897. He obtained his B.S. degree from Greenville College in 1919. He received his A.B. degree in 1921, M.S. degree in 1923, and Ph.D. in 1924, all at the University of Illinois. His major field was Bio-Chemistry. He taught in the following institutions: University of Louisville, Johns Hopkins University, and University of Michigan. In 1935 he was appointed Senior Pharmacologist in the Food and Drug Administration and Principal Pharmacologist in 1936, and Chief of the Division of Pharmacology in 1941. Dr. Calvery was noted for his fair, just, and rapid decisions in pharmaceutical matters.

HARRY D. GRIGSBY

Born April 26, 1884 in Cuba, Illinois. He received his B.S. degree from the University of Illinois in 1906. From 1907 to 1912 he was a chemist and inspector in testing for the C.R.I. & P. Railroad. From 1912 to 1914, when he was with the Illinois State Food Commission, he was a chemist. He came to the Bureau of Chemistry in 1914. With the exception of the period from 1918 to 1924 when he was with the Ohio State Dairy and Food Department, he was a chemist at various places for the old Bureau of Chem-

istry and the Food and Drug Administration. At the time of his death he was Chief Chemist of the Philadelphia Station. Mr. Grigsby was noted for his ability as a chemist and for his friendly and helpful attitude towards his associates.

(Miss) Janet E. Harris

Born April 18, 1918. Died on June 30, 1945. She obtained her B.A. degree in 1939 in Bacteriology at the Ohio State University and did graduate work at Columbia. After some experience as a medical technologist in hospitals, she joined the Food and Drug Administration in July, 1943, as a Junior Chemist.

George M. Johnson

Mr. Johnson was born in Chicago, Illinois, on November 25, 1904. He died March 5, 1946. He received his B.S. degree from the University of Chicago in 1926. After some graduate work at the University of Chicago, he worked as a commercial chemist before joining the Food and Drug Administration in 1932 as a Junior Chemist. In his association with this department he was stationed in Minneapolis and St. Louis.

Albert E. Plumb

Born December 12, 1885, at North Bangor, New York, he died March 9, 1945. Mr. Plumb received his B.S. degree in 1909 from Syracuse University. He was Chief Chemist in New York City from 1919 to 1920. After chemical experience in industry he was appointed in 1929 as chemist in the Food and Drug Administration. He was stationed first at Buffalo, then at San Francisco, and later, St. Louis.

Hugo Reed

Born July 13, 1899, in Astoria, Oregon. He received his B.S. degree in 1922, and M.S. in 1924, from the University of Oregon. He was appointed to the Bureau of Chemistry in 1925 and stationed at New York City. He was then transferred to the Seattle Station in 1926 where he spent the rest of his life.

State Chemists

J. D. Turner

He was born March 18, 1887. He died January 1, 1946. Mr. Turner received his B. of Pedagogy degree in 1898 and a B. of L. in 1910. In 1906 he became Head of the Department of Feeding Stuffs of the Kentucky Agricultural Experiment Station. In 1939 Feeds and Fertilizer Departments were combined and he became head of the entire control service. He was noted for his counsel and consulting advice to the control officers.

CHARLES C. CATHCART

Born Januray 2, 1865. Died December 9, 1945. He obtained his B.S. degree at Rutgers in 1886. In 1889 he obtained his M.S. degree. Mr. Cathcart was an Assistant Chemist with the firm of Austin & Wilbur from 1890 to 1892. He was Chief Chemist for Lister Agricultural Chemical Works of Newark, New Jersey, from 1893 to 1907. He was Chief Chemist in charge of inspection and analysis of commercial fertilizers and feeding stuffs from 1907 until his death.

ANTHONY P. KERR

Born May 4, 1892 in Gils, Mississippi. He died January 9, 1946. Mr. Kerr obtained his B.S. at Mississippi State College in 1906 in Chemistry. He had a long association with the Louisiana Experiment Station and State Department of Agriculture and Immigration. Much of this time was spent assisting in the administration of the fertilizer and feed laws.

M. S. ANDERSON, *Chairman*
R. P. THOMAS

REPORT OF THE COMMITTEE ON NOMINATIONS

President: J. O. Clarke, Food and Drug Administration, Chicago, Ill.

Vice-President: G. H. Marsh, Director, Chemistry Div., Montgomery, Ala.

Secretary-Treasurer Emeritus: W. W. Skinner, Kensington, Md.

Secretary-Treasurer: Henry A. Lepper, Food and Drug Administration, Washington, D. C.

Additional Members of the Executive Committee: L. S. Walker, Agricultural Experiment Station, Burlington, Vt.; W. A. Queen, Food and Drug Administration, Washington, D. C.; H. A. Halvorson, State Chemist, St. Paul, Minn.; W. H. Ross, Washington, D. C.

G. G. FRARY
H. J. WICHMANN
W. H. MACINTIRE, *Chairman*

Approved.

REPORT OF THE COMMITTEE ON RESOLUTIONS

Whereas, the 60th annual meeting of the Association of Official Agricultural Chemists has brought the 62nd year in the history of the Association to a successful conclusion; and

Whereas, the large attendance has benefited from the program;

Be it resolved, that the Association express its appreciation to Dr. W. H. Ross for his services during his extended term of office, for his excellent presidential address presenting a concise summary of crop increases during

the past eight decades, and for the able manner in which he has conducted the meeting; to Mr. J. O. Clarke for his assistance as Vice President; to Mr. Henry A. Lepper for the organization of this meeting, and for efficient supervision of the day-by-day affairs of the Association as its Secretary-Treasurer; to all other officers, section chairmen, committeemen, referees, and collaborators, for their services; and to all State and Federal units for their interest in sending representatives to this meeting.

Be it further resolved, that our Secretary extended the thanks of the Association to the managers of the Shoreham Hotel for the facilities made available for its meetings, and for their courtesy and cooperation during a period of stress in their organization.

Be it further resolved, that the Association commend Dr. H. J. Fisher and the members of his committee for their success in the arduous task of compiling the 6th Edition of the Book of Methods.

Be it further resolved, that the Secretary express to Hermann C. Lythgoe, G. S. Fraps, and W. F. Hand, former presidents of the Association, our regrets that retirement from State employ terminates their long and valuable services to the Association in many official positions, and the hope that they may return to join our distinguished Corps of Elder Statesmen at future meetings; and that the Secretary express to Mrs. Marion Lapp Otis, who served in so many capacities under the inadequate title of Associate Editor, the gratitude of the Association for her tactful guidance in the past, and the hope that she may again participate actively in its affairs.

COLIN W. WHITTAKER
JOHN B. SMITH

Approved.

CONTRIBUTED PAPERS

TITRATION METHOD FOR POTENTIAL SOIL NEUTRALIZING POWER OF BLAST FURNACE SLAGS[1]

By C. J. SCHOLLENBERGER (Associate in Agronomy, Ohio Agricultural Experiment Station, Wooster, Ohio)

A simple and reliable chemical test for checking the potential soil neutralizing power of blast furnace slag is needed, in view of the increasing interest in its use as a liming material. The method of assay by dissolving a weighed sample in a measured volume of standard acid, boiling off carbon dioxide and back-titrating excess acid with standard alkali to the phenolphthalein end point, which indicates satisfactorily the calcium carbonate equivalent of limestone, etc. (1), has caused much complaint when applied to slags.

Efforts have been made to improve the titration procedure for this application. Crane noted that taking the end point at the methyl orange yellow, somewhat over *p*H 4, instead of the phenolphthalein pink at about *p*H 8, caused high results which he attributed to the reaction of some alumina as a base (5). Titrations to *p*H 4.6 with the glass electrode as indicator (2), or to 5.2 with bromocresol green, are stated to give approximate values by Shaw, who has reported extensive studies of methods which may be used for evaluating silicate liming materials (7). The latest specifications for a tentative method call for a glass electrode titration to end point *p*H 4.8 (3). The glass electrode has obvious disadvantages, and the usual procedure of titration with a color indicator is rather unsatisfactory because dark impurities are likely to be present or to precipitate as the titration progresses.

The fundamental difficulty in back-titrating an acid solution of an aluminosilicate like slag is that the silica, alumina, etc., precipitate in gelatinous form when the titration has progressed to a point in the range *p*H 3–4. The precipitate interferes with the titration, both mechanically and chemically. It is of acid nature and is capable of absorbing a part of the alkali added to complete the titration. As may be seen from the glass electrode titration curve for an oxidized solution of slag in excess acid (Figure 1), the characteristics of a satisfactory titration are lacking when the titration is continued to the end in the presence of the precipitate. The curve shows no steep slope, hence there is no sharp end point. The indication for the calcium carbonate equivalent at the phenolphthalein end point falls short of the value calculated from the known analysis of the slag, 89.2 per cent, by about 9 per cent. But if the titration is interrupted at a

[1] Contribution from the Department of Agronomy, Ohio Agricultural Experiment Station, Wooster, Ohio.

point just a little short of complete precipitation, before the *p*H value has reached 4, and the precipitate is filtered out, the titration curve of the filtered solution follows its previous course briefly and then steepens, becoming almost vertical and so indicating a good end point at a point corresponding to expectations from the known analysis of the slag. This end point coincides with the appearance of the phenolphthalein pink at about *p*H 8.

In contrast with the simple assay of a limestone for potential soil neu-

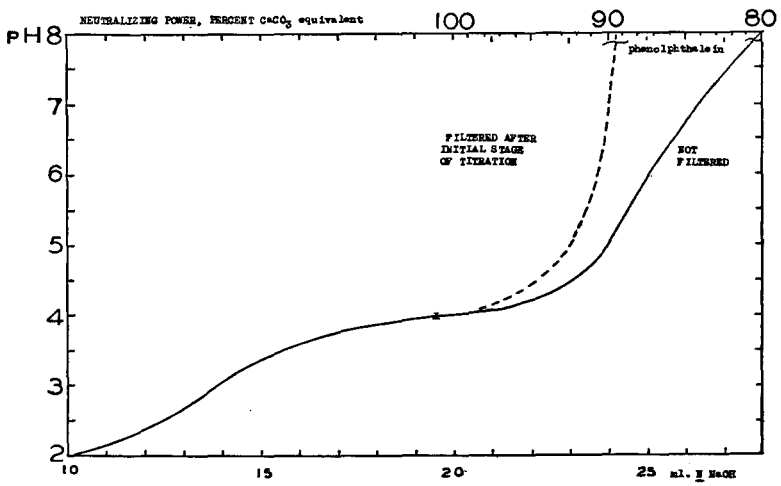

FIG. 1.—Glass electrode titration of slag solution (2 g in 60 ml *N* HCl, in 200 ml volume, with and without filtration)

tralizing power, there are numerous difficulties and sources of error when a similar procedure is applied to a slag.

1. Solution is more difficult, mainly from the tendency for gelatinous or solid silica to separate, cementing the sample into a mass or otherwise protecting it from further attack by the acid.

2. Most of the silica and alumina of the slag go into solution and these constituents precipitate in gelatinous form during the titration, causing interference as previously described. As with incomplete solution of the sample, this source of error tends toward low results for the calcium carbonate equivalent.

3. Slags contain considerable amounts of ferrous and manganous oxides and sulfides, and sometimes metallic iron, which dissolve in the standard acid and act as strong bases, thus being included in the lime equivalent determined by the titration in the usual manner. But these constituents of the slag contribute little or nothing to the soil neutralizing power. Insofar as they may be active in the soil they would eventually change to non-basic forms; the iron and manganese would be oxidized to the insoluble higher oxides, and the sulfur to neutral sulfates, with consumption of equivalent lime bases. These reactions would tend toward high indica-

tions for the calcium carbonate equivalent, but they are insufficient for full compensation.

To improve the usual procedure, the following modifications seem practical:

1. Grind the sample finely in preparation, and dissolve it in an adequate excess of cold standard hydrochloric acid, with sufficient agitation to keep the particles in suspension. Solution in cold acid reduces the tendency for silica to separate, and usually results in more rapid and complete attack upon the slag than is possible with immediate heating.

2. Interrupt the titration at a suitable point, just short of complete formation of the aluminosilicic acid precipitate, filter, and take a large aliquot of the filtrate to avoid washing the precipitate, and complete the titration thereon, in the absence of much precipitate. The comparatively small requirement of alkali to complete the titration of the aliquot is multiplied by a suitable factor to make it correspond to the entire sample. It is known that the precipitate retains practically no acid in basic salts (7), and any other error incident to this novel treatment should be insignificant in comparison with that which is thereby avoided.

3. Collect in a measured volume of standard alkali the hydrogen sulfide evolved during solution of the slag. Add hydrogen peroxide to this alkaline solution to oxidize the absorbed sulfur to sulfate, thus neutralizing a corresponding part of the alkali used. Add this mixture containing the undetermined excess of alkali and hydrogen peroxide to the acid solution of the sample, for the effect of the residual alkali and to oxidize the ferrous and manganous salts derived from the sample, so that the acid which was consumed by these elements will be included in the titration of excess acid. Count all the alkali so used as a part of that required for the initial stage of titrating excess acid; whatever part of it may have been neutralized by sulfur will appear as the same amount additional to complete the titration, and the correction for sulfur is thus applied automatically without being determined separately. All the iron and manganese, as well as the sulfides, are thus excluded from the calcium carbonate equivalent determined; the value should correspond closely to that calculated from calcium plus magnesium oxides minus sulfur equivalents indicated by the complete analysis.

METHOD

Transfer a 1.667 g sample (ground so that all will pass a No. 100 sieve) to a 250 ml wide-neck volumetric (Pyrex phosphate) flask. Wash down with 25 (or 50) ml water and swirl to disperse the sample immediately. Run down the side, without mixing, 75.00 ml *N* (or 50.00 ml 1.5 *N*) hydrochloric acid. Insert in the flask a Pyrex culture tube of suitable size, containing 5.00 ml *N* sodium hydroxide and a little water, and without delay attach to the flask a rubber stopper carrying a 50 ml pipet or bulbed tube for a trap to scrub vapors, as shown in Figure 2. Swirl the acid mixture in the flask promptly and frequently until as much as practical of the sample has been dissolved by the cold acid **(a)**. Heat and boil at a moderate rate for 5 minutes, or longer if the alkali solution in the trap becomes yellow and the color deepens with continued boiling **(b)**. Loosen the stopper before allowing the assembly to cool. Remove and lay aside the pipet. Withdraw the culture tube, rinsing back adhering acid. To the cooled alkali solution in the tube add 2 ml of 30 per cent hydrogen peroxide. Stir the mixture with the stem of the pipet used in the trap, draw up, and shake to insure oxidation of all sulfur absorbed. Pour the alkali solution from the tube into the cooled flask and follow with rinsings from tube and pipet **(c)**. Start the titration with *N* sodium hydroxide without any indicator; when

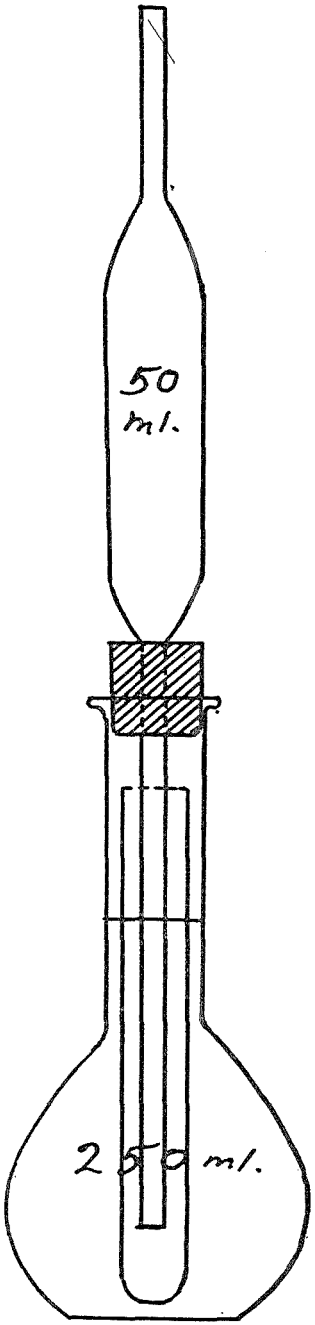

FIG. 2.—Flask and trap for dissolving slag samples.

a total of about 35 ml has been added, the yellow color of ferric iron should have disappeared and the mixture will be filled with a gelatinous precipitate, usually light colored and not disappearing on shaking **(d)**. At this point, stop and test by dropping in a bit of methyl orange paper. At the first test, the paper should turn red or reddish orange, indicating insufficient alkali addition. Thereafter add the *N* sodium hydroxide in 1 ml increments, shaking well and testing with a fresh paper each time, until the paper turns orange yellow on striking the solution, and becomes nearly pure yellow after floating on the surface for 15 seconds **(e)**. Each addition of *N* sodium hydroxide, up to the last, should have increased the amount of permanent precipitate noticeably. Dilute with water to within 2 ml of the 250 ml mark, stopper, and shake thoroughly, then release the pressure developed by decomposing peroxide. After standing for a minute, the precipitate should settle in the neck of the flask, leaving a narrow layer of clear solution. If this does not appear, add 1 ml more *N* sodium hydroxide and shake again **(f)**. Note the total volume of *N* sodium hydroxide used to this point, including the 5.00 ml initially put in the trap (x ml). Dilute to the 250 ml mark and shake well at intervals for a minute or so. Pour on a dry fluted 18½ cm paper with rapid filtering characteristics, such as S. & S. American, No. 588. Transfer 200 ml of the clear and colorless filtrate to a flask, if necessary boil to expel carbon dioxide and cool, add 0.5 ml phenolphthalein indicator solution and 1–2 g barium sulfate, X-ray quality. Complete the titration with *N* sodium hydroxide to a faintly pink color, permanent on shaking, and easily visible against the background of suspended white barium sulfate after any dark gelatinous precipitate has settled on standing for a few seconds. If no dark precipitate has appeared when the end point is reached, add 3–4 drops of peroxide before concluding that the titration is finished **(g)**. Note the volume of *N* sodium hydroxide used in the second stage of the titration (x′ ml), which should be 2–6 ml for optimum results. With sample weight and solutions as specified, the per cent calcium carbonate equivalent to potential soil neutralizing power is equal to $3(75.00 - x - 1.25\,x')$.

NOTES ON METHOD

(a) With samples ground to pass a No. 100 sieve, from about one minute to more than two hours has been required for apparently complete decomposition by cold acid in the procedure described. After agitation until danger of setting to a mass is past, the assembly may be left to stand overnight for complete attack. As with limestones, resistance to acid has been noted to vary directly with per cent MgO, but to be much greater with unquenched (air-cooled), than with quenched (water- and dry-granulated), slags of about the same MgO contents.

(b) Slags differ with respect to evolution of their sulfur as hydrogen sulfide in this procedure. Johnson (6) states that not all the sulfur can be obtained from some steels by the evolution method. With air-cooled slags in particular, a part of the sulfur appeared as the element and sometimes could be seen as a yellow sublimate in a cooler part of the apparatus. Being slowly volatile with steam, most of this can be driven into the trap by longer boiling, but in some instances only about two-thirds of the total could be recovered. Elemental sulfur causes the alkali solution in the trap to become yellow. Finely ground slag samples which had been kept air-dry have shown in some instances up to a third of their total sulfur as sulfate soluble in dilute hydrochloric acid without added oxidant.

(c) Oxidation of the absorbed sulfur will be complete only in alkaline solution.

(d) The iron is completely precipitated in the initial stage of the titration, but not as red-brown ferric hydroxide, as shown by the light color. In the absence of dark dirt and graphite, etc., the precipitate is usually a very light tan or greenish; the latter tint may result from a blue component sometimes visible, probably a ferrocyanide. It seems likely that the trivalent elements (including manganese in the presence of peroxide, to some extent) may form a complex with the silica. If the latter is largely dehydrated and made insoluble during soln of the sample, the iron is not precipitated as described but forms a red hydrosol persisting beyond the phenolphthalein end point of a titration. Silica in soln and the oxidizing effect of excess peroxide are essential factors in the titration.

TABLE 1.—*Producer's analyses of slags and $CaCO_3$ equivalents calculated therefrom and determined by titration*

SLAG	NATURE[a]	PRODUCER'S ANALYSES							CALCULATED $CaCO_3$ EQUIVALENT			INDICATED BY TITRATIONS
		SiO_2	Al_2O_3	FeO	MnO	CaO	MgO	S	CaO+MgO	−S =	NNP[b]	NNP[b]
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
A	WG	34.9	10.6	0.8	1.4	48.8	2.4	1.3	93.1	4.2	88.9	87.5 ±0.8
B	WG	35.7	10.8	3.2	0.3	45.6	2.7	1.5	88.1	4.7	83.4	83.1 ±1.4
C	WG	35.1	12.0	2.7	1.4	40.3	7.0	0.9	89.3	2.9	86.4	87.8 ±1.3
D	DG	37.1	13.7	0.9	0.5	43.9	1.9	1.1	83.1	3.4	79.7	80.6 ±1.0
E	DG	34.7	11.1	0.9	0.9	40.7	9.4	1.0	96.0	3.1	92.9	94.1 ±0.8
F	AC	34.5	13.2	1.6	2.1	37.7	9.1	1.4	89.9	4.5	85.4	83.7 ±2.2
G	AC	34.0	13.4	1.4	1.4	41.7	5.4	1.1	87.8	3.4	84.4	84.6 ±0.3
H	AC	37.4	12.7	1.6	1.2	39.6	4.7	0.8	82.4	2.5	79.9	81.2 ±0.2
I	AC	38.6	14.4	1.6	0.3	40.4	3.1	1.1	79.8	3.4	76.4	75.1 ±0.7
J	AC	35.9	12.9	1.7	1.4	41.3	4.1	1.7	83.9	5.2	78.7	81.5 ±2.3
K	AC	34.4	12.6	0.6	1.2	41.5	5.5	1.5	87.8	4.6	83.2	85.7 ±1.6
											83.6	84.0 ±1.1
Standard sample, av.[c]		36.3	10.6	0.6	0.5	43.5	7.2	1.6	95.6	5.0	90.6	89.7 ±0.2
Av. Deviation		± 0.4	0.5	0.2	0.1	0.3	0.4	0.1	1.1	0.3	1.1	

[a] WG = water granulated (quenched); DG = dry granulated; AC = air cooled in mass.
[b] NNP = net neutralizing power for soil; lime bases minus sulfur as equivalent per cent $CaCO_3$.
[c] Average percentages and average deviations therefrom reported by 35 producer's laboratories. Data from 4 titrations by the author with the method described are shown in the last column.

(e) Thin filter paper is soaked in methyl orange indicator soln and dried. It should have an orange color. In acid soln and unprotected, methyl orange is quickly yellowed, then bleached, by peroxide; when absorbed on paper, it is sufficiently protected to be useful. Tests on the filtered solns ranged *p*H 3.1–4.1 by the glass electrode, the average value and deviation shown by 33 separate solns being *p*H 3.6 ±0.1.

(f) Settling clear as described seems to be associated with rapid filtration in the next step.

(g) The peroxide originally added may have been entirely decomposed previously, so none is left to promote the precipitation of manganese as dioxide. But not infrequently, the final soln has been practically free of manganese, nearly all of that in the sample having fallen with the precipitate in the first titration stage. It must be precipitated at some time for the acid initially required for its solution to be included in the back-titration. Hydrogen peroxide should cause no appreciable error in the titration when the end point is taken as the first faint pink of phenolphthalein. According to Britton (4), pK_a for hydrogen peroxide is 12, therefore at *p*H 8 it is only 0.01 per cent dissociated and the entire amount used for a determination would consume only about 0.01 ml. *N* alkali. But at higher *p*H the buffer property is marked and a considerable addition of *N* sodium hydroxide is required to intensify the phenolphthalein color.

DISCUSSION

Although there are sources of error, the foregoing method is believed to be about as accurate as the usual producer's slag analysis. Aside from the great difficulty in getting all of some samples into solution in acid and the uncertainty of the sulfur correction because not all the sulfur may be evolved as hydrogen sulfide, the expedient of filtering off the precipitate before the titration is completed is only a partial remedy for what is believed to be the principal source of error—that is, absorption of alkali by the precipitate. The titration still has to be completed in the presence of part of the precipitate, so there will still be some tendency toward low indications for the calcium carbonate equivalent, to some extent compensatory to the plus error from the imperfect sulfur correction. The producer's analyses in Table 1 may be taken as the standard whereby to judge the accuracy of the values indicated by the titrations. The agreement of averages is satisfactory, and with individual samples there is no instance in which the difference would ordinarily be considered of much significance in evaluating a liming material. The average deviation from averages of the triplicated titrations is 1.1 per cent calcium carbonate equivalent; this is the same as calculated from data reported for a standard sample of slag analyzed in 35 producer's laboratories. It is concluded that for all practical purposes this titration method should serve as well as the standard method for slag analysis for evaluation as a liming material. In most instances, the expense would prohibit application of the standard method of slag analysis to samples of small lots as delivered, but in this respect the titration is more practical.

SUMMARY

A modification of the usual method for determining the calcium carbonate equivalent of liming materials by acidimetric titration, whereby more accurate indications may be obtained in evaluating blast furnace

slags, is described. Trials with a variety of blast furnace slags from the Pittsburgh district showed satisfactory agreement with calcium carbonate equivalents calculated from total calcium plus magnesium oxides minus sulfur equivalents shown by the producer's analyses.

ACKNOWLEDGMENT

Mr. W. M. Shaw of the Tennessee Station has studied the evaluation of slags by titration, and generously lent the manuscript describing his work in advance of publication (7). Mr. H. T. Williams of The Standard Slag Co., Youngstown, Ohio, has cooperated by supplying slag samples of known history and analyses, and general information about blast furnace slags. For this and other help, thanks are expressed.

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FURTHER STUDIES ON OFNER'S METHOD FOR THE DETERMINATION OF INVERT SUGAR

By EMMA J. MCDONALD AND ANNE L. TURCOTTE
(National Bureau of Standards, Department of Commerce, Washington, D. C.)

I. INTRODUCTION

It has long been recognized that copper solutions of low alkalinity are better suited for the analysis of small amounts of invert sugar in the presence of sucrose than are those of higher alkalinity. The method devised by Ofner (1) for the determination of invert sugar in refined sugars has been subjected to critical study by Jackson and McDonald (2). These authors recommended the use of the Ofner method with some modifications. These included the substitution of an ordinary asbestos gauze plate for the metal gauze, thus insuring more uniform heating and the elimination of the "flame spot." The precision of the method was greatly increased by acidifying with acetic acid before the addition of iodine and subsequent addition of hydrochloric acid. Thus, the rapid oxidation of cuprous chloride in acid solution was eliminated.

Jackson and McDonald confined their experiments to sugar samples containing from 5 to 25 mg of invert sugar, which amounts to 0.05 to 0.25 per cent when a 10-gram sample is used. In the present investigation the precision of the method was determined with invert sugar alone, and with sugar samples containing less than 0.05 per cent invert sugar. The results further confirm the validity of the modified Ofner method for the analysis of refined sugars.

Experience has shown that invert sugar can be determined more accurately alone than when in the presence of sucrose. Therefore, it seemed advisable to base the results on the invert-sugar values and to apply a correction for the increased reduction due to the action of the sucrose.

II. ANALYTICAL PROCEDURE

Invert sugar used in these experiments was prepared by weighing equal quantities of levulose and dextrose and by the hydrolysis of pure sucrose. Identical analytical results were obtained from invert sugar prepared by the two procedures. The sucrose and dextrose used were National Bureau of Standards Standard Samples Nos. 17 and 41, respectively; the levulose was purified by repeated crystallization from aqueous alcohol solution. Iodine of a normality of 0.0323 was used except when 5 mg or less of invert sugar was determined, in which case 0.00323 N iodine was employed. All results were expressed in terms of the 0.0323 N solution. The concentration of the iodine solution was determined by volumetric titration against thiosulfate of the same normality.

The reagents and procedure used were those recommended by Jackson and McDonald (34.47–8 of the 1945 *Book of Methods*) with the following exceptions: First, the thiosulfate solution was repeatedly standardized (3, 4) against pure copper or a copper sulfate solution in which the copper had been determined electrolytically. Second, during the boiling the mouth of the flask was closed by means of a small inverted beaker or glass bulb.

III. REDUCING POWER OF INVERT SUGAR

Forty-five analyses were made in which quantities of invert sugar varying from 0.5 to 25 mg were determined. The data were treated by the method of averages in order to obtain a mathematical relation between the iodine consumed and the invert sugar present. It was found that when not more than 5 mg of invert sugar was present, the following linear relation existed:

$$S = 0.177 + 1.0124\, I, \tag{1}$$

where S = milligrams of invert sugar, and I = milliliters of 0.0323 N iodine consumed. Above this range the following quadratic equation was found to apply:

$$S = 0.817 + 0.855\, I + 0.005117\, I^2. \tag{2}$$

Table 1 records a series of determinations of known quantities of invert sugar in which the sugar is calculated from the iodine consumed by means of equation (1) or (2).

Ofner found that 1 ml of 0.0323 *N* iodine was equivalent to 1 mg of in-

TABLE 1.—*Determination of invert sugar*

0.0323 *N* IODINE	INVERT SUGAR			0.0323 *N* IODINE	INVERT SUGAR		
	PRESENT	DETERMINED FROM I_2 CONSUMED BY MEANS OF EQUATION (1) OR (2)	DIFFERENCE		PRESENT	DETERMINED FROM I_2 CONSUMED BY MEANS OF EQUATION (1) OR (2)	DIFFERENCE
ml	*mg*	*mg*	*mg*	*ml*	*mg*	*mg*	*mg*
0.33	0.5	0.5[a]	0.0	9.94	10.0	9.8	−0.2
0.51	0.7	0.7	0.0	12.62	12.5	12.4	−0.1
0.82	1.0	1.0	0.0	12.71	12.5	12.5	0.0
0.81	1.0	1.0	0.0	12.72	12.5	12.5	0.0
1.27	1.5	1.5	0.0	12.59	12.5	12.4	−0.1
1.75	2.0	2.0	0.0	12.55	12.5	12.35	−0.15
1.76	2.0	2.0	0.0	15.14	15.0	14.9	−0.1
1.76	2.0	2.0	0.0	15.52	15.0	15.3	+0.3
2.21	2.5	2.4	−0.1	15.00	15.0	14.8	−0.2
2.75	3.0	3.0	0.0	15.29	15.0	15.1	+0.1
2.78	3.0	3.0	0.0	17.18	16.9	17.0	+0.1
3.01	3.4	3.2	−0.2	17.63	17.5	17.5	0.0
3.80	4.0	3.9	−0.1	17.47	17.5	17.3	−0.2
3.86	4.0	4.1	+0.1	20.04	20.0	20.0	0.0
4.05	4.4	4.3	−0.1	19.85	20.0	19.8	−0.2
4.90	5.0	5.1	+0.1	20.11	20.0	20.1	+0.1
4.94	5.0	5.2	+0.2	22.52	22.5	22.7	+0.2
4.93	5.0	5.2[b]	+0.2	22.40	22.5	22.5	0.0
7.46	7.5	7.5	0.0	22.31	22.5	22.4	−0.1
7.48	7.5	7.5	0.0	24.52	25.0	24.9	−0.1
7.43	7.5	7.45	−0.05	24.85	25.0	25.2	+0.2
10.01	10.0	9.9	−0.1	24.72	25.0	25.1	+0.1
10.09	10.0	10.0	0.0				

[a] The first seventeen values under this heading were calculated by the equation: $S = 0.177 + 1.0124\ I$, where S = milligrams of invert sugar, and I = milliliters of 0.0323 *N* iodine consumed.

[b] The last twenty-eight values under this heading were calculated by the equation: $S = 0.817 + 0.855\ I + 0.005117\ I^2$, where S = milligrams of invert sugar, and I = milliliters of 0.0323 *N* iodine consumed.

vert sugar. The changes in the procedure, however, make the results more reproducible and thus the analytical results warrant the more precise mathematical treatment. Table 2 correlates the iodine consumed and the invert sugar present. It is calculated by means of the above equations and is intended for practical application.

IV. EFFECT OF SUCROSE ON THE REDUCING POWER OF INVERT SUGAR

It has been shown by many investigators that sucrose, even after careful purification, has a reducing effect on alkaline copper solutions. Thus, in using any method employing these reagents the question arises whether to make a correction for the reduction of pure sucrose or to express the

TABLE 2.—*Iodine-invert sugar equivalents*

0.0323 *N* IODINE	FRACTIONAL MILLIMETERS OF 0.0323 *N* IODINE									
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	MILLIGRAMS OF INVERT SUGAR									
ml										
0	—	—	—	0.48	0.58	0.68	0.78	0.89	0.99	1.09
1	1.19	1.29	1.39	1.49	1.59	1.70	1.80	1.90	2.00	2.10
2	2.20	2.30	2.40	2.51	2.61	2.71	2.81	2.91	3.01	3.11
3	3.21	3.31	3.41	3.52	3.62	3.72	3.82	3.92	4.02	4.12
4	4.23	4.33	4.43	4.53	4.63	4.73	4.83	4.94	5.04	5.14
5	5.24	5.33	5.41	5.50	5.59	5.67	5.77	5.86	5.95	6.04
6	6.13	6.22	6.32	6.41	6.50	6.59	6.68	6.78	6.87	6.96
7	7.05	7.15	7.24	7.33	7.43	7.52	7.61	7.70	7.80	7.89
8	7.98	8.08	8.17	8.27	8.36	8.45	8.55	8.64	8.74	8.83
9	8.93	9.02	9.12	9.21	9.31	9.40	9.50	9.59	9.69	9.79
10	9.88	9.98	10.07	10.17	10.26	10.36	10.45	10.55	10.65	10.74
11	10.84	10.94	11.03	11.13	11.23	11.33	11.42	11.52	11.62	11.71
12	11.81	11.91	12.01	12.11	12.21	12.30	12.40	12.50	12.60	12.70
13	12.80	12.90	12.99	13.09	13.19	13.29	13.39	13.49	13.59	13.69
14	13.79	13.89	13.99	14.09	14.19	14.29	14.39	14.49	14.59	14.69
15	14.79	14.89	14.99	15.10	15.20	15.30	15.40	15.50	15.60	15.71
16	15.81	15.91	16.01	16.11	16.21	16.32	16.42	16.52	16.63	16.73
17	16.83	16.93	17.04	17.14	17.24	17.35	17.45	17.55	17.66	17.76
18	17.86	17.97	18.07	18.18	18.28	18.39	18.49	18.60	18.70	18.81
19	18.91	19.01	19.12	19.22	19.33	19.44	19.54	19.65	19.75	19.86
20	19.96	20.07	20.18	20.28	20.39	20.49	20.60	20.71	20.81	20.92
21	21.03	21.14	21.24	21.35	21.46	21.56	21.67	21.78	21.89	22.00
22	22.10	22.21	22.32	22.43	22.54	22.64	22.75	22.86	22.97	23.08
23	23.19	23.30	23.41	23.52	23.62	23.73	23.84	23.96	24.07	24.18
24	24.28	24.39	24.50	24.61	24.72	24.83	24.95	25.06	25.17	25.28
25	25.39	25.50	25.61	25.72	25.85	25.95	26.06	26.17	26.28	26.39

results on the basis of invert sugar alone. If a correction is to be made, the reducing power of very pure sucrose must be determined. Bates and Jackson (5), using Soldaini's reagent (a copper carbonate-bicarbonate solution) found a reduction of copper corresponding to 0.006 per cent invert sugar content in pure sucrose. Kraisy, using a copper carbonate tartrate solution, found 0.007 per cent, while Sandera and Merceo, and the New York Sugar Trade Laboratory, using the same method (6), found 0.003 per cent as the invert content of sucrose. Ofner reports that 10 g of sucrose reduces copper equivalent to 1 ml of 0.0323 *N* iodine, or 0.01 per cent invert sugar. Jackson and McDonald (2) found that 10 g of sucrose (NBS Standard Sample No. 17) reduced copper equivalent to 1.1 ml of 0.0323 *N*

iodine. The same value was found in the present investigation, a reduction corresponding to 0.013 per cent invert sugar.[1]

If the sucrose hydrolyzes during the determination, it would be expected that methods employing different boiling times and solutions varying in *p*H would give varying results. It is known that the alkaline solutions,

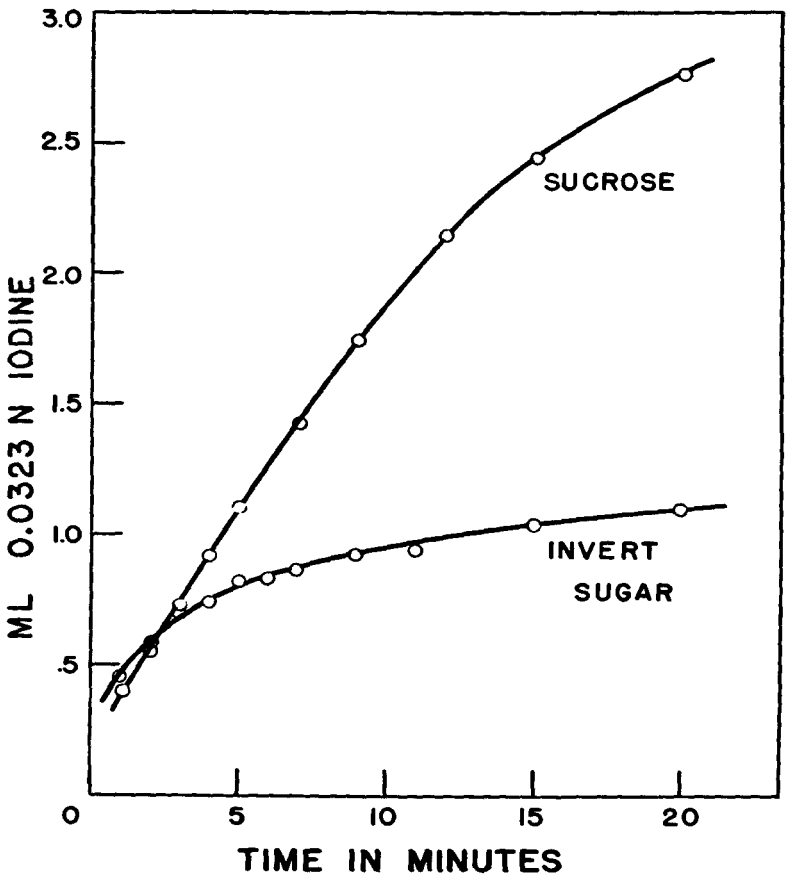

FIG. 1.—The effect of the time of boiling on the determination of invert sugar and sucrose.

such as Soxhlet's, have a hydrolytic effect on sucrose, to such an extent that they are not adaptable to the determination of refined sugars. A series of analyses was made in which 1 mg of invert sugar and a sample of sucrose containing 1.3 mg of apparent invert sugar were analyzed by the

[1] Repeatedly recrystallized and freshly prepared sucrose samples occasionally gave values as low as 0.006 per cent of invert sugar when analyzed by this method.

method under consideration, using 1 to 20 minutes boiling time. The results are shown in Figure 1. It is concluded from the difference in the slopes of the curves at the 5-minute boiling time (the conditions specified in the method) that the reducing effect of sucrose is caused, at least in part, by substances formed during the analysis rather than those present in the original sample. These results also show that when sucrose is present small variations in the boiling time will have a greater effect on the amount of copper reduced than when invert sugar alone is present.

Many investigators, using different copper solutions under varying conditions, have studied the effect of sucrose on the reducing power of invert sugar. Ofner reported that, irrespective of the relative amounts of sucrose

TABLE 3.—*Reducing power of sucrose alone and with added invert sugar*

SUGAR SAMPLE	10 GRAMS OF SUCROSE		MILLIGRAMS OF INVERT SUGAR ADDED									
			1		2		3		4		5	
	I_2	INVERT SUGAR BY FORMULA	PRESENT	FOUND	PRESENT	FOUND	PRESENT	FOUND	PRESENT	FOUND	PRESENT	FOUND
	mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
1	1.61	1.8	2.8	2.8	3.8	3.4	4.8	4.6	5.8	5.9	6.8	7.2
2	1.07	1.3	2.3	2.3	—	—	4.3	4.4	5.3	5.5	6.3	6.5
3	1.71	1.9	2.9	2.9	3.9	3.8	4.9	5.1	5.9	6.0	6.9	7.2
4	1.07	1.3	2.3	2.3	3.3	3.3	4.3	4.2	5.3	5.3	—	—
5	1.66	1.9	2.9	2.8	3.9	3.4	4.9	4.6	5.9	5.9	6.9	7.2
6	1.06	1.25	2.25	2.2	3.25	3.25	4.25	4.3	5.25	5.4	6.25	6.6
7	5.98	6.2	7.2	7.2	8.2	8.4	9.2	9.6	—	—	—	—

and invert sugar present, ten grams of the former or one milligram of the latter reduced copper equivalent to one milliliter of 0.0323 *N* iodine.

In Table 3 the results of the analyses of seven samples of sucrose, alone and with known amounts of added invert sugar, are tabulated. It was found that, when the total invert sugar content of 10 g of sucrose was not more than about 5 mg, the effect of the sucrose and added invert sugar seemed to be additive. It is therefore recommended that for sucrose samples containing 0.05 per cent or less of invert sugar the iodine used be converted to invert sugar by use of Table 2 and the results thus obtained reported as apparent invert sugar. Alternately, a correction amounting to 1.1 ml of 0.0323 *N* iodine for each 10 grams of sucrose may be subtracted from the titration in order to compensate for the reduction of pure sucrose. In the latter case the results would be expressed as invert sugar and would not include the apparent invert sugar caused by the reducing action of the pure sucrose.

For amounts of invert sugar above 5 mg, the table published by Jackson and McDonald (2) is more convenient and accurate.

V. SUMMARY AND CONCLUSIONS

Ofner's method, as modified by Jackson and McDonald, has been used for the determination of invert sugar and a table giving the invert sugar equivalents of the iodine consumed has been prepared. It has been shown that this is a convenient and reliable method for the analysis of high-grade refined sugars. The reducing effect of pure sucrose is also discussed.

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DETERMINATION OF THE TITRATABLE ACIDITY OF MILK*

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The titratable acidity in fresh milk, commonly calculated as lactic acid, has been shown to be due largely to phosphates, carbon dioxide, and casein, rather than to lactic acid (1, 2). The outstanding points to consider in making the titration are: one, the type and strength of alkali; two, the color at the end point; three, the amount of indicator; and, four, the question of diluting the milk (3). The first two points will not be considered here, since caustic soda is the alkali commonly used in this country, and the first permanent pink color is the logical end point of the titration. Since lactic acid is practically absent from fresh milk, it seems logical to use a dilution of the milk and an amount of the indicator which will give the lowest apparent acidity. In other words, we should seek those conditions that will require the least amount of alkali to reach the end point. In this study 17.6 ml of milk was used for each titration and carbon dioxide-free water was used for all dilutions. The alkali was 0.1 *N* NaOH and the indicator was a 1% solution of phenolphthalein in 95% neutral alcohol. The *p*H at the end point was measured by means of a Beckman Laboratory Model *p*H Meter, using the glass and calomel electrodes.

Rather widely differing amounts of indicator have been recommended for use in titrating milk, although phenolphthalein is universally used. In studying the effect of concentration of indicators we have found that an increase in the amount of indicator decreases the *p*H of the solution at the

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end point and, therefore, lowers the titration figure. This is shown in Table 1.

Table 1 indicates that the minimum amount of alkali is required when 2 ml of the 1% phenolphthalein are used for 17.6 ml of milk diluted with an equal volume of water. Larger amounts of indicator appear to have little effect. This is, therefore, apparently the optimum concentration of indicator. Two ml of the indicator solution in 35 ml of diluted milk is equivalent to approximately 54 milligrams of phenolphthalein per 100 ml of milk solution. H. Barkworth (4) concluded that the necessary concentration of the indicator to obtain the desired color change at *p*H 8.3 was 80 milligrams per 100 ml of undiluted milk. Barkworth worked only with undiluted milk. However, it was found that with less indicator and with some dilution of the milk, the first definite pink color is

TABLE 1.—*Effect of increasing quantity of indicator*

DILUTION	1% PHENOLPHTHALEIN	0.1 *N* NaOH	*p*H AT END POINT	ACID AS LACTIC
	ml	*ml*		*Per cent*
1+1	0.5	2.79	8.25	.140
1+1	1.0	2.70	8.25	.135
1+1	1.5	2.60	8.15	.130
1+1	2.0	2.50	8.04	.125
1+1	2.5	2.45	8.02	.123
1+1	3.0	2.42	8.02	.121
1+1	4.0	2.45	8.02	.123

reached at *p*H 8.0–8.1, instead of at 8.3. Kolthoff and Laitinen (5) give the *p*H range of the phenolphthalein pink in 90% alcohol as 8.0–9.8.

Døvle (6) points out that a given color intensity implies a constant *p*H and a constant concentration of color ions. This concentration is dependent upon the amount of color present as well as upon the *p*H. This is equally true with cloudy or opaque fluids, but in such cases it will require a greater concentration of color ions to give the same intensity. Therefore, since the opacity of milk is lessened by dilution, the end point should be reached at lower *p*H when lesser concentration of indicator is used.

The effect of varying the concentration of indicator was also tested on a solution of pure lactic acid. This solution was prepared as follows: Zinc lactate was prepared from zinc oxide and lactic acid. It was re-crystallized several times, and then dried over sulfuric acid. Enough zinc lactate to produce a solution containing 0.12% of lactic acid was quickly weighed and dissolved in carbon dioxide-free water. The zinc was precipitated with H_2S and the excess H_2S was eliminated by passing natural gas through the solution. The gas was then eliminated by aspiration with CO_2-free air and the solution made up to volume.

Portions of this solution were then titrated with standard NaOH, using varying amounts of indicator. Titration was carried to the first appearance of pink color in this water-clear solution. Immediately upon completion of each titration the *p*H was read. The results shown in Table 2 demonstrate that concentration of phenolphthalein indicator is without effect upon the end point in a water solution of pure lactic acid.

TABLE 2.—*Results with increasing concentration of indicator*

DILUTION	1% PHENOLPHTHALEIN	0.1 *N* NaOH	*p*H AT END POINT	ACID AS LACTIC
	ml	*ml*		*Per cent*
1+1	0.2	2.41	8.48	.120
1+1	0.6	2.40	7.96	.120
1+1	1.0	2.40	8.13	.120
1+1	1.6	2.40	8.21	.120
1+1	2.0	2.40	8.34	.120
1+1	2.6	2.40	8.21	.120
1+1	3.4	2.40	8.02	.120
1+1	4.0	2.40	8.27	.120

The effect of dilution of the milk was studied and the results are shown in Tables 3 and 4. The unit volume of milk, 17.6 ml, was diluted with the indicated volumes of water, freed of carbon dioxide.

TABLE 3.—*Results of increasing dilution of milk on titratable acidity*

DILUTION	1% PHENOLPHTHALEIN	0.1 *N* NaOH	*p*H AT END POINT	ACID AS LACTIC
	ml	*ml*		*Per cent*
1+0	2.0	2.89	8.00	.145
1+1	2.0	2.50	8.06	.125
1+2	2.0	2.29	8.10	.115
1+3	2.0	2.19	8.12	.110
1+4	2.0	2.05	8.15	.103
1+5	2.0	2.02	8.16	.101
1+8	2.0	1.89	8.16	.095
1+10	2.0	1.75	8.13	.088

Since the results in Table 1 had shown 2 ml of 1% indicator solution to be the correct amount to use in diluted milk, that volume was used in the titrations recorded in Table 3. The volume of alkali needed to reach the end point was found to decrease as the dilution increases. This is due to the fact that the end point is more easily perceptible as the opacity of the milk solution decreases. However, it is not practicable to use too great a dilution. Therefore, further study was made on dilutions up to one plus three, varying the amount of indicator. These titrations are shown in Table 4.

Results tabulated in Tables 3 and 4 indicate that dilution has a marked effect upon titratable acidity and that 80 milligrams of phenolphthalein per 100 ml of milk solution are not necessary. Table 4 indicates that a 1 plus 2 dilution requires less alkali than does a 1 plus 1 dilution. It shows also that when a 1 plus 2 dilution is used approximately the same results

TABLE 4.—*Dilution up to 1 plus 3, with varying amount of indicator*

DILUTION	1% PHENOLPHTHALEIN	0.1 *N* NaOH	*p*H AT END POINT	ACID AS LACTIC
	ml	*ml*		*Per cent*
1+1	1.0	2.45	8.16	.123
1+1	2.0	2.40	8.09	.120
1+1	3.0	2.35	8.10	.118
1+2	0.5	2.45	8.30	.123
1+2	0.5	2.35	8.24	.118
1+2	1.0	2.30	8.26	.115
1+2	1.0	2.31	8.26	.116
1+2	2.0	2.19	8.15	.110
1+2	2.0	2.19	8.15	.110
1+2	1.0	2.29	8.12	.115
1+2	1.0	2.20	8.07	.110
1+2	2.0	2.15	8.12	.108
1+2	2.0	2.10	8.08	.105
1+2	3.0	2.15	8.09	.108
1+2	3.0	2.09	8.06	.105
1+3	0.5	2.29	8.32	.115
1+3	0.5	2.28	8.32	.114
1+3	1.0	2.15	8.16	.108
1+3	1.0	2.17	8.21	.109
1+3	2.0	2.09	8.16	.105
1+3	2.0	1.99	8.15	.100

are obtained with either 2 or 3 ml of indicator. When 2 ml of 1% indicator are used, further dilution does not affect results significantly.

In Table 5 is shown a comparison between results by the present tentative method and those obtained by using greater dilution of sample and higher concentration of indicator.

It may be noted from Table 5 that the present method required 0.5 ml more alkali to reach the end point, and that the end point *p*H is 0.1 unit higher. This increase in alkali corresponds to 0.02 per cent acid as lactic.

Considering the above series of titrations with varying dilutions of milk and concentrations of indicator, it is recommended that a milk solution prepared by diluting 17.6 ml of milk with twice its volume of car-

bon dioxide-free water be titrated to the first definite pink color with 0.1 *N* NaOH, using as indicator 1 ml of 2% phenolphthalein in neutral alcohol. This added dilution, as compared with the present official method (7) so affects the salt-alcohol complex as to require less alkali to reach the end point; and it consequently gives a lower apparent acidity. Further dilution of the milk sample, or concentration of the indicator, causes only

TABLE 5.—*Comparison of present (tentative) method and the method proposed*

DILUTION	1% PHENOLPHTHALEIN	0.1 *N* NaOH	*p*H AT END POINT	ACID AS LACTIC
	ml	*ml*		*Per cent*
1+1	0.5	2.70	8.31	.135
1+1	0.5	2.69	8.32	.135
1+1	0.5	2.68	8.32	.134
1+1	0.5	2.70	8.34	.135
1+1	0.5	2.70	8.34	.135
	2% PHENOLPHTHALEIN			
	ml			
1+2	1.0	2.20	8.22	.110
1+2	1.0	2.21	8.20	.111
1+2	1.0	2.22	8.18	.111
1+2	1.0	2.25	8.18	.113
1+2	1.0	2.20	8.18	.110
1+2	1.0	2.21	8.18	.110

slight variation in the *p*H, and has but little effect on the volume of alkali needed to reach the end point.

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DETERMINATION OF PHOSPHORIC ACID IN FERTILIZERS IN THE PRESENCE OF ORGANIC MATTER*

By H. R. ALLEN and LELAH GAULT (Kentucky Agricultural Experiment Station, Lexington, Ky.)**

The present A.O.A.C. methods for phosphoric acid in presence of considerable organic matter (3) specify (a) evaporation of sample with magnesium nitrate solution, ignition, and digestion in hydrochloric acid, or (b) digestion of sample with mixtures of sulfuric acid, nitric acid, and potassium nitrate.

The writers have found that evaporation with magnesium nitrate is time consuming and cumbersome, and that low results may be obtained in some cases. One reported (1) that digestion with sulfuric acid of a sample of primary potassium phosphate, and of Bureau of Standards Rock Phosphate sample No. 56, gave high results with the volumetric method, and

TABLE 1.—*Phosphoric acid content of organic materials, using different methods of solution. Results in percent* P_2O_5

SAMPLE	SOLUTION OF SAMPLE BY—		
	$H_2SO_4+KNO_3$	HNO_3+HClO_4	HNO_3+HCl
Cottonseed meal	3.15	3.08	.95
Soybean oil meal	1.58	1.55	.90
Castor pumace	1.75	1.73	.88
Sardine meal	6.33	6.28	6.16
Fish meal	8.33	8.25	8.05
Tobacco stems	1.16	1.20	1.16

theoretical results with the gravimetric method. This paper presents results of further investigation of the problem including results by an alternate method using perchloric acid in digestion.

Richardson (5) attributed high results when precipitation is made in the presence of sulfates to the formation of an acidic ammonium sulfomolybdate. This was confirmed by Falk and Suguira (2). In 1929 the A.O.A.C. volumetric method was changed (4) to require precipitation in the presence of sulfates at room temperature, with shaking. All determinations reported in this paper were made in this manner.

EXPERIMENTAL

Solutions of various organic materials were prepared in 250 ml volumetric flasks by digestion with (a) 30 ml nitric and 5 ml hydrochloric

* This investigation, made in connection with a project of the Kentucky Agricultural Experiment Station, is published by permission of the Director.
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acid, (b) 20 ml sulfuric acid with the addition of potassium nitrate and (c) 20–30 ml nitric acid, evaporating nearly to dryness, cooling, adding 5 ml of 70% perchloric acid and boiling on the hotplate, nearly to dryness. Phosphoric acid was determined by the volumetric method in 0.2 gram aliquots. Results are shown in Table 1.

TABLE 2.—*Effect of different methods of solution and different treatments of aliquot solution on percent P_2O_5*

SAMPLE	ALIQUOT SOLUTION NEUTRALIZED		ALIQUOT SOLUTION NOT NEUTRALIZED	
	NO HNO_3 ADDED TO MOLYBDATE	HNO_3 ADDED TO MOLYBDATE	NO HNO_3 ADDED TO MOLYBDATE	HNO_3 ADDED TO MOLYBDATE
	20 ml H_2SO_4+KNO_3			
Cottonseed meal* plus No. 6959	9.13	9.05	9.00	8.90
Soybean oil meal plus No. 6959	8.98	8.88	8.83	8.75
Castor pumace plus No. 6959	8.93	8.75	8.70	8.63
Sardine meal plus No. 6959	9.43	9.30	9.30	9.18
Fish meal plus No. 6959	9.65	9.50	9.50	9.40
—No. 6959†	8.88	8.73	8.60	8.63
	20 ml HNO_3+5 ml $HClO_4$			
Cottonseed meal plus No. 6959	8.90	8.88	8.90	8.88
Soybean oil meal plus No. 6959	8.75	8.73	8.75	8.73
Castor pumace plus No. 6959	8.73	8.70	8.70	8.68
Sardine meal plus No. 6959	9.15	9.13	9.15	9.10
Fish meal plus No. 6959	9.40	9.38	9.40	9.35
—No. 6959†	8.64	8.58	8.63	8.58
	30 ml HNO_3+5 ml HCl			
Cottonseed meal plus No. 6959	8.70	8.63	8.63	8.63
Soybean oil meal plus No. 6959	8.65	8.60	8.60	8.60
Castor pumace plus No. 6959	8.70	8.63	8.65	8.63
Sardine meal plus No. 6959	9.10	9.08	9.08	9.05
Fish meal plus No. 6959	9.40	9.35	9.38	9.33
—No. 6959†	8.68	8.60	8.63	8.60

* Each sample of mixture consists of 0.2 grams of the organic material and 1.8 grams of No. 6959, a 3-9-6 fertilizer.
† 1.8 grams.

The phosphoric acid content of mixtures of some of these organic materials, and of a 3-9-6 fertilizer which contained no organic matter, was determined after digestion by the various procedures listed, using 2-gram samples composed of 0.2 gram of an organic material and 1.8 gram of the mixed fertilizer. Digestion was made in 250 ml volumetric flasks and a 25 ml aliquot, corresponding to 0.2 gram, was taken for analysis. The

A.O.A.C. volumetric method (4) was used with the exception noted as to neutralization of sample aliquots.

Results from samples where sulfuric acid digestion was used varied considerably according to whether the sample aliquots were neutralized or not and whether or not nitric acid was added to the molybdate solution.

TABLE 3.—*Effect of different treatments of the aliquot solution on mg P_2O_5 from water solution of KH_2PO_4* (H_2SO_4 used)*

SAMPLE	.025 GM ALIQUOT (½ PERCENTAGE)	.05 GM ALIQUOT (¼ PERCENTAGE)
Water solution alone	13.00	26.10
8.6 ml (1+3) H_2SO_4 added, then neutralized	13.50	27.15
3 ml (1+3) H_2SO_4 added, then neutralized	13.65	—
3 ml (1+3) H_2SO_4 added, not neutralized	13.45	27.00
7 ml (1+3) H_2SO_4 added, not neutralized	13.30	26.82
Water solution plus 3 gms $(NH_4)_2SO_4$. No NH_4NO_3 used	—	27.15
Theory	13.04	26.08

* Theory 52.16 per cent P_2O_5.

TABLE 4.—*Effect of different treatments of the aliquot solution on mg. P_2O_5 from water solution of KH_2PO_4* (HNO_3 used)*

SAMPLE	.025 GM ALIQUOT (½ PERCENTAGE)	.05 GM ALIQUOT (¼ PERCENTAGE)
Water solution alone	13.00	26.10
2 ml (1+1) HNO_3 added, then neutralized	13.10	26.10
3 ml (1+1) HNO_3 added, not neutralized	13.00	26.10
7 ml (1+1) HNO_3 added, not neutralized	13.00	26.15
Water solution plus 1 gm $(NH_4)_2SO_4$	—	26.85
Water solution plus 3 gms $(NH_4)_2SO_4$	—	27.10
Theory	13.04	26.08

* Theory, 52.16 per cent P_2O_5.

Table 2 shows a comparison between the 3 methods of solution, between aliquots neutralized and not neutralized, and between added nitric acid in the molybdate solution and no added nitric.

The phosphoric acid content of reagent grade primary potassium phosphate, dried over sulfuric acid, was determined from water solution alone, from water containing varying amounts of (1+3) sulfuric acid, or of (1+1) nitric acid, both neutralized and not neutralized. Results are given in Tables 3 and 4.

DISCUSSION OF RESULTS

Digestion of the samples of organic materials in nitric-hydrochloric acid obtained only one-third to one-half the phosphorus from cottonseed meal,

soybean oil meal, and castor pumace. Digestion in these acids gave from 95 to 100 per cent of the phosphorus in sardine meal, fish meal, and tobacco stems. Digestion of all organic materials in nitric-perchloric acids gave results equal to, or only slightly lower than, those from solutions of sulfuric acid.

Results on the mixtures of organic materials with mixed fertilizer were too low from nearly all the digestions with nitric-hydrochloric acid; but it is believed the nitric-perchloric acid digestion obtained nearly all the phosphorus in such mixtures, except in one or two instances where results may have been about 0.1 per cent low. After evaporation of the perchloric acid digestion to a low volume, it may be necessary to add 50 ml of water and bring to a boil to insure complete solution, and this was not always done. Results from the perchloric acid digestions varied but little with the different treatments of the aliquot solutions.

The amounts of phosphoric acid obtained from sulfuric acid digestions varied about 0.25 per cent from high to low, according to the treatment of the aliquot solutions, in the following order: solution neutralized and no nitric acid added to molybdate; solution neutralized and nitric acid added to molybdate; solution not neutralized and no nitric acid added to molybdate; solution not neutralized and nitric acid added to molybdate (5 ml nitric acid to 100 ml molybdate). If one assumes the true phosphoric acid content of the mixtures to be that of the mixed fertilizer alone using a nitric-hydrochloric acid or perchloric acid digestion, plus that of the organic material using the sulfuric or perchloric acid digestion, the first treatment in the descending series gave high results, the second treatment (the official A.O.A.C. method) was slightly high for mixtures containing cottonseed meal or soybean oil meal, and the third treatment (solution not neutralized and no acid added to molybdate) is probably the most satisfactory one using sulfuric acid in digestion.

The amounts of (1+1) NH_4OH necessary to neutralize the aliquots from the various digestions were: from sulfuric acid digestion, 9.7 to 10.2 ml; from nitric-perchloric acid digestion, 0.4 to 0.7 ml; from nitric-hydrochloric acid digestion, 2.0 to 2.9 ml. The use of bromothymol blue indicator to determine neutrality of the aliquot solutions was more satisfactory than litmus paper.

No difficulty was experienced with the nitric-perchloric acid digestion when the digestion was first carried to a low volume with nitric acid before adding the perchloric acid. The volumetric flask* used has a relatively short neck which aids evaporation and the bottom of the flask is slightly concave, which helps to prevent bumping at low volume.

Use of less sulfuric acid in the digestion was tried. Digestion with 30 ml nitric acid and 5 ml sulfuric acid with added potassium nitrate was

* Arthur Thomas catalog No. 5524.

satisfactory in destroying organic matter but phosphoric acid results were higher than when the larger volume of sulfuric acid was used.

Precipitation of the sulfate with barium eliminated the influence of the sulfate ion but results could not always be duplicated, possibly because of the occlusion of some phosphate by the very heavy barium sulfate precipitate. This precipitate was excessive, even when only 5 ml of sulfuric acid was used.

Ammonium sulfate, 1 to 3 grams, added to the aliquot solution from non-sulfuric acid digestions, gave higher results, similar to those obtained from the use of sulfuric acid.

Results with reagent grade primary potassium phosphate in the presence of sulfate gave higher results alone than when organic matter was also present.

SUMMARY

If sulfuric acid is used for solution of the samples of fertilizer containing organic material, high results for phosphoric acid by the volumetric method are obtained unless the ammonium phosphomolybdate is precipitated in strongly acid solution. Highest results are obtained when the aliquot solution is neutralized as in the official procedure and no nitric acid is added to the molybdate solution. It appears that precipitation without neutralization of the aliquot solution, and without addition of acid to the molybdate solution, is preferable to the official procedure.

Solution of sample in nitric acid followed by digestion with perchloric acid seems promising, but in some instances results may be slightly low.

Determination of phosphoric acid in the presence of sulfate gives higher results for inorganic components alone than in mixtures containing organic material. Use of 1 to 3 grams of ammonium sulfate in the aliquot solution from other than sulfuric acid digestion, gives high results.

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THE RECOVERY OF DDT FROM CANNED FOODS AND ITS STABILITY DURING PROCESSING*

By C. J. TRESSLER, JR. (National Canners Association, Washington, D. C.)

In dealing with the analytical problems involved in the determination of DDT residues in canned foods one is confronted with several problems. These consist of: (1) the selection of a suitable analytical method, (2) the choice of a method for extraction of DDT from the foods, and (3) the determination of the stability of DDT under the processing conditions during the canning procedure.

In selecting an analytical method for determining DDT, methods involving the determination of organic chlorine were avoided because there would be danger of contamination from excessive inorganic chlorides. There is also the possibility of partial breakdown of DDT, with the organic chlorine still confined within the can. Further, both labile and total chlorine would have to be determined and the ratio of these two calculated. Accordingly the method selected for this work was the Schechter and Haller colorimetric method[1] as modified by Clifford.[2] With this method, the amounts of both p,p'- and o,p-DDT may be determined, and if other nitrated products are present in the final determination, their presence may be confirmed by readings taken at 450 millimicrons. In some of the work both DDT isomers were being determined and in this case the two-color system was followed; but in most of the experiments only the p,p'-isomer was involved and readings were made at a wave length of 610 millimicrons. A Coleman Universal Spectrophotometer with a one-half inch cell was used.

For extraction of DDT residues from canned foods a method was sought which would be rapid enough for canning plant laboratories to use as a check for DDT in finished products. Since DDT is highly soluble in benzene and very insoluble in aqueous solutions, it was reasoned that if a food item were blended with a definite volume of benzene in a Waring Blendor, essentially all of the DDT would be in the benzene. An aliquot of the benzene would then contain a known percentage of the DDT present. Very stable emulsions are formed when most foods are blended with benzene, and recovery of a representative benzene aliquot is difficult or impossible when the emulsions are directly centrifuged. Of the products worked with, tomato juice was the only one from which a 70 per cent aliquot was obtainable. With other products, centrifuging released only small amounts of benzene. It was found that if anhydrous sodium sulfate was added to the emulsion in the blendor and the mixture further blended for ca 2 min-

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 14–16, 1946.

[1] Schechter, M. S., and Haller, H. L., *J. Am. Chem. Soc.*, 66, 2129 (1944).

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utes, the benzene was fairly well released by centrifuging. From 50 to 85 per cent of the benzene was recoverable from the products tested. These

TABLE 1.—*Recovery of p,p'-DDT from tomato juice (emulsion broken with varying amounts of anhydrous sodium sulfate)*

TOMATO JUICE	DDT PRESENT	BENZENE	Na_2SO_4	BENZENE ALIQUOT	DDT IN ALIQUOT	RECOVERY	
ml	*mg*	*ml*	*gm*	*ml*	*mg*	*mg*	*Per cent*
200	0.5	100	0	60	0.30	0.290	97
200	0.5	100	50	60	0.30	0.285	95
200	0.5	100	100	60	0.30	0.288	96
200	0.5	100	200	55	0.28	0.252	92

TABLE 2.—*Recovery of p,p'-DDT from various unprocessed and processed foods*

ITEM	ADDED p,p'-DDT PRESENT IN BENZENE ALIQUOT	PROCESS	RECOVERY	
	milligrams		*mg*	*Per cent*
Strained Peaches	0	0	0.02	—
	0.60	0	0.52	87
	0.60	0	0.51	85
	0	0	0.01	—
	0.70	0	0.62	89
	0.70	0	0.53	76
	0	25 @ 212°F.	0.01	—
	0	25 @ 212°F.	0.01	—
	0.70	0	0.54	77
	0.70	0	0.53	76
	0.52	25 @ 212°F.	0.25	48
	0.70	25 @ 212°F.	0.36	51
Strained Applesauce	0	25 @ 212°F.	0.01	—
	0	25 @ 212°F.	0.01	—
	0.68	0	0.42	62
	0.62	0	0.38	61
	0.54	25 @ 212°F.	0.23	43
	0.64	25 @ 212°F.	0.27	42
Green Beans	0	0	0.01	—
	0.56	0	0.44	79
	0.56	0	0.45	75

included strained peaches, strained pears, applesauce, and green beans. The amount of sodium sulfate necessary to break down the emulsions was found to be about one-half of the weight of food sampled. This amount has no effect on the DDT, as shown by recovery tests where varying amounts

of sodium sulfate were added to tomato juice containing DDT. The per cent of DDT recoverable ranged from 97 with no sodium sulfate added, to 96 with 50 per cent by weight of sodium sulfate, and to 92 per cent when equal weights of the juice and the salt were used. In the last case, the reduction to 92 per cent may not be significant because the mixture was too thick to handle properly.

This system of DDT extraction was used to determine the recoveries

TABLE 3.—*Recovery of p,p'-DDT processed in water and in potassium acid phthalate buffer*

ITEM	p,p'-DDT ADDED	PROCESS	RECOVERY	
	mg		*mg*	*Per cent*
		A. Sealed in Pyrex Test Tubes		
Water	0.58	0	0.58	100
	0.58	0	0.57	98
	0.58	1 hr. @ 212°F.	0.57	98
	0.58	2 hrs. @ 212°F.	0.54	93
	0.58	4 hrs. @ 212°F.	0.54	93
Buffer	0.70	0	0.71	101
*p*H = 4.0	0.70	0	0.70	100
	0.70	2 hrs. @ 212°F.	0.70	100
	0.70	4 hrs. @ 212°F.	0.70	100
		B. Sealed in Tin Cans		
Water	0.70	0	0.64	91
	0.70	1 hr. @ 212°F.	0.63	90
	0.70	2 hrs. @ 212°F.	0.60	86
	0.70	4 hrs. @ 212°F.	0.56	80
Buffer	0.70	0	0.64	91
pH = 4.0	0.70	1 hr. @ 212°F.	0.59	84
	0.70	2 hrs. @ 212°F.	0.56	80
	0.70	4 hrs. @ 212°F.	0.55	79

possible in various foods. These are included in Table 2 and range from as high as 89 per cent in strained peaches to as low as 61 per cent in the case of applesauce. No explanation is offered for these variances. Accordingly, when a product is to be tested for an unknown amount of DDT, it is suggested that this same product containing no DDT be run as a blank, and also with DDT added as a control.

When testing methods for DDT in canned foods, one is confronted with the necessity of knowing whether DDT is stable at the temperatures encountered during processing. To determine the stability of DDT during processing conditions, known amounts of the p,p'-isomer were processed

in distilled water, in potassium acid phthalate solution, and in certain foods. The DDT was then extracted and determined.

To several Pyrex test tubes each containing 5 ml of water, and to several more each containing 5 ml of potassium acid phthalate buffer at pH = 4.0, a solution of DDT in alcohol was added. The tubes were sealed and processed at 212°F. for varying times up to 4 hours. The tubes were then opened and the DDT extracted with ether and determined. The same experiment was then repeated, using 100 ml of water or buffer in

TABLE 4.—*Recovery of p,p' and o,p-DDT from unprocessed and processed tomato juice (no. 1 cans)*

NO.	DDT PRESENT IN BENZENE ALIQUOT		PROCESS	RECOVERY		PER CENT RECOVERY	
	o,p-	p,p'-		o,p-	p,p'-	o,p-	p,p'-
	mg						
1	0	0	0	0.02	0.01	—	—
2	0.70	0	0	0.73	—	104	—
3	0.70	0	20′ @ 212°F.	0.26	—	37	—
4	0	0	0	0.02	0.01	—	—
5	0	0.70	0	—	0.68	—	97
6	0	0.70	0	—	0.68	—	97
7	0	0.70	20′ @ 212°F.	0.14*	0.22	—	31
8	0	0.70	20′ @ 212°F.	0.14*	0.22	—	31
9	0.30	0.70	0	0.34	0.63	113	90
10	0.30	0.70	0	0.36	0.64	120	91
11	0.30	0.70	20′ @ 212°F.	0.19**	0.23	63**	33
12	0.30	0.70	20′ @ 212°F.	0.19**	0.22	63**	31

* Disintegration products give an apparent amount of o,p-isomer.
** Subtracting apparent o, p- amount, under (*), of 0.14 mg. leaves an actual amount of 0.05 mg, or 17 per cent recovery.

tin cans. The amounts of DDT recovered are shown in Table 3. Little destruction was encountered when using Pyrex tubes. With the tin cans, destruction amounted to about 20 per cent at the end of four hours both in water and in phthalate buffer.

DDT was also added to strained peaches, and to applesauce, which were then sealed in tin cans, processed for the recommended times, then removed, and the DDT extracted with benzene using the extraction method previously mentioned. The results are included in Table 2 and show recoveries of about 50, and slightly over 40, per cent of the DDT added to the respective products. This would indicate destructions of about 27 per cent, and close to 20 per cent, respectively, referring the recovered values to the control values obtained at the same time.

Tomato juice containing either o,p- or p,p'-DDT, or both isomers to-

gether, was processed and the residual DDT determined in like manner. These results are listed in Table 4. The first three determinations show that only 37 per cent of the o,p-isomer is recoverable after processing. In determinations 4 through 8 only 31 per cent of the p,p'-isomer was recoverable. These also indicate that processing changed the color system, so that the two-color colorimetric method showed an apparent amount of o,p-isomer present. The presence of this foreign color leads to the suspicion that some of the DDT breakdown products may have been nitrated. In determinations 9 through 12, recovery tests were made for both the added isomers. The p,p'-isomer was recovered in the same percentage as when the isomer was by itself, but the o,p-isomer had what might seem to be a 63 per cent recovery. However, subtracting 0.14 mg (the amount of o,p-falsely read in runs 7 and 8) from the apparent amount recovered in numbers 11 and 12 gives an "adjusted" value of only .05 mg of o, p-isomer, a recovery of 17 per cent.

SUMMARY AND CONCLUSIONS

1. Clifford's adaptation of the Schechter colorimetric method for DDT has been found applicable for determining DDT residues in canned foods.

2. A rapid method for extracting DDT from canned foods is presented.

3. Although DDT was found to be stable to heat in water and in acid buffer in Pyrex containers, and only slightly decomposed in these media in tin cans, it was decomposed to a greater degree when processed with various foods.

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DETERMINATION OF COPPER IN FOODS BY A TWO-COLOR DITHIZONE METHOD*

By C. A. GREENLEAF (National Canners Association, Washington, D. C.)

Various methods utilizing dithizone for determining small amounts of copper have been described. In these, dithizone is used either for preliminary isolation of copper or for final determination. Interference of other metals is not difficult to overcome, since copper can be extracted at a low enough *p*H to avoid interference by such metals as nickel and cobalt (1, 2); other possible interferences, such as bismuth, silver, and mercury,

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may be eliminated by an acidified potassium iodide wash (2, 4). There are, however, certain features which have led to divergence in choice of techniques in application of dithizone to the determination of copper.

One of these features is the effect of electrolyte strength on the rate of extraction of copper. It has been shown (3) that from a simple acid solution copper may be readily extracted at a *p*H well below 1, provided an excess of dithizone is used. If there is a considerable concentration of salts, such as results from neutralization of an acid digest, the extraction is greatly retarded, and protracted shaking is required to obtain equilibrium. Thus, in the method described by Bendix and Grabenstetter (2), a shaking time of 10 minutes is specified, making the use of a mechanical shaker a practical necessity if many determinations are to be made. It has been shown (1) that the rate of extraction is greatly increased by making the extraction at *p*H 3.0–3.3, instead of 2.3 as specified by Bendix and Grabenstetter, and without incurring risk of interference by nickel or cobalt. If much bismuth is present some may be extracted, but it is readily eliminated by an acidified potassium iodide wash. In the work reported here, the same was found to be true of zinc and cadmium, if present in large amounts.

Another point of divergence is the use of a one-color or mixed-color procedure for final determination. In the mixed-color method as usually applied, that is, with a uniform concentration of total dithizone, the sensitivity is much less than that characteristic of one-color dithizone methods. This is a point in favor of the one-color procedure, which is embodied in the Bendix and Grabenstetter method (2). Objection to this procedure is based on the fact that removal of excess dithizone by an alkaline wash changes variable portions of the violet-colored keto copper dithizonate into the yellowish-brown enol tautomer. This has been pointed out by Sandell (5) and by Morrison and Paige (6). The latter authors use the basic procedure of Bendix and Grabenstetter, adapted to Mojonnier fat-extraction flasks, but omit the final ammonia wash. Another objection to the one-color method is the persistent tendency for alkaline solutions to take up from glassware traces of zinc, which then appears as copper in the result.

It has seemed to the author that the sensitivity of the mixed color procedure might be increased by varying the concentration of dithizone so that only a moderate excess is present even when the amount of copper is small. In order for such a method to be accurate and not too complicated, some simple means of taking account of the variable excess of dithizone is needed. Such a technique is offered by the scheme of colorimetric analysis described by Knudson, Meloche, and Juday (7), and since applied by others (8, 9, 10, 11, 12) to the analysis of two-component colored systems. It consists essentially in determining the optical density at two wave lengths at which exist the greatest differences in density of the two components.

In the method described here, copper is extracted with a large excess of dithizone in carbon tetrachloride, from a sample solution adjusted to *p*H ca 3.1 and containing potassium iodide. The sample solution is removed and the dithizone solution shaken with acidified potassium iodide solution to remove possible traces of silver, mercury, bismuth, zinc, or cadmium. The dithizone solution is then transferred to a second separator, where the dithizone is decomposed with bromine water and the copper is transferred to 0.01 *N* HCl, the oxidized dithizone being discarded. After washing the aqueous layer with carbon tetrachloride and removing the residue of solvent by aspiration, the solution is buffered by *p*H 2.9 by adding acid potassium phthalate solution, a little sodium bisulfite solution is added, and the solution is then ready for extraction with the dithizone solution used for final determination. The latter is added in portions from a buret, with shaking after each addition, until the color indicates an excess. Enough carbon tetrachloride is then added to give a volume of 15 ml and after final shaking the dithizone extract is drawn off and its optical density is measured at 520 mμ and at 625 mμ. Reference to a calibration chart or equation gives the amount of copper.

METHOD

APPARATUS

A photoelectric spectrophotometer, Coleman Universal, with cuvettes of square cross section, ½ inch cell depth, No. ST-10-S.

Separators, Squibb short-stemmed, 250 ml and 125 ml.

Clean glassware with hot HNO_3. Grease lower stopcocks of separators with white petrolatum and do not use brass chains.

REAGENTS

Water.—Redistilled from Pyrex Glass, for all dilutions and for making solutions not specially purified.

Nitric acid.—Redistil C.P. acid from Pyrex.

Hydrochloric acid, 6 N.—Dilute conc. HCl 1+1 and distil from Pyrex. Prepare 1 *N* and 0.01 *N* solns.

Ammonium hydroxide.—Distil in Pyrex from concentrated soln into redistilled water (600 ml water per liter of original reagent) surrounded by ice bath. Final volume about 900 ml.

Sulfuric acid, C.P.—This is the least controllable source of blank; usually contains 1 or 2 parts per million of copper.

Carbon tetrachloride, C.P.—Shake thoroughly with ca 10 per cent NaOH soln, dehydrate with anhydrous Na_2SO_4 and redistil over CaO, rejecting the first few per cent containing moisture. In reclaiming used CCl_4, add NaCN to the NaOH soln. Shake dithizone soln containing bromine with acidified bisulfite solution before reclamation.

Dithizone.—Dissolve 30 mg of the solid in 20 ml $CHCl_3$ and shake out with two portions, 100 ml and 50 ml, of dilute NH_4OH (1:100). Discard $CHCl_3$. Extract ammonia solns in series with small portions of CCl_4 until extracts are practically colorless. Combine and filter ammonia solns, add 10 ml $NaHSO_3$ soln and a slight excess of 6 *N* HCl. Extract dithizone with several portions of CCl_4 and make extract

up to 1 liter. Dilute 300 ml of this to 500 ml with CCl_4. Store both solns, designated as 25 mg/l and 15 mg/l respectively, in refrigerator in dark bottles, under a layer of 0.1 *M* sulfurous acid ($NaHSO_3+HCl$).

Ammonium citrate soln.—Dissolve 150 gm citric acid in 600 ml H_2O and make just alkaline to cresol red (alkaline range) with NH_4OH, using spot plate; add 10 ml NH_4OH in excess and dilute to 1 liter. Extract with dithizone soln in $CHCl_3$ until extracts are green, then with $CHCl_3$ until extracts are colorless, and finally with CCl_4. Filter.

Potassium iodide soln, 10%.—Dissolve 50 gm KI and make up to 500 ml. Place in separatory funnel with $CHCl_3$ soln of dithizone. Add NH_4OH dropwise with shaking until dithizone begins to transfer to aqueous phase. Extract with further portions of dithizone until extracts are green, then add HCl dropwise until dithizone is precipitated from aqueous phase. Extract with CCl_4 and filter. If free iodine appears on standing, discharge with dilute $Na_2S_2O_3$ or $NaHSO_3$ added dropwise.

Potassium iodide soln, 2%.—To 100 ml of 10% potassium iodide soln, add 6 ml of 1 *N* HCl and dilute to 500 ml. If free iodine appears on standing, decolorize as directed for the 10% soln.

Acid potassium phthalate soln, 0.1 M.—Dissolve 10.2 gm of the salt and dilute to 500 ml. Extract with dithizone soln in CCl_4 and finally with CCl_4. Filter.

Sodium bisulfite soln, 3.2%.—Purify with dithizone as directed for acid potassium phthalate. Prepare fresh frequently.

Copper standard.—Dissolve 0.2000 g of c.p. Cu wire or foil in 15 ml HNO_3 (1+4), covering with a watch-glass and warming to complete solution. Boil to expel fumes, cool, and dilute to 200 ml. Prepare suitable dilutions to obtain a soln containing 3 micrograms per ml. Transfer 8, 16, 24, and 32 ml of this to 200 ml volumetric flasks, add 2 ml 1 *N* HCl to each and make up to volume with water. A 25 ml aliquot then contains 3, 6, 9, or 12 micrograms of Cu in 0.01 *N* HCl.

PREPARATION OF SAMPLE

Prepare the sample by wet combustion with HNO_3 and H_2SO_4, either on macro or micro scale. If macro wet combustion is used, take an amount of sample estimated to contain 20–100 micrograms of Cu and use 20 ml H_2SO_4. When the digest remains yellow and does not char on evolution of SO_3 fumes, add a little more HNO_3 and 5 ml of 60–70% $HClO_4$. Continue heating until $HClO_4$ is expelled. Cool, add 50 ml H_2O and heat to fumes again. Cool and dilute to 200 ml in a volumetric flask. If micro digestion apparatus is used, reduce the quantities of sample and acids accordingly, and dilute with 10–20 ml H_2O before transfer to the separator. Run blanks with all samples.

ISOLATION OF COPPER

In a 250 ml short-stemmed separator place 10 ml of ammonium citrate soln and add the sample solution from micro digestion or a 20 ml aliquot from macro digestion. Dilute to ca 85 ml, add 0.5 ml bromophenol blue indicator, and NH_4OH dropwise until the bluish tinge of the indicator appears, then 1 *N* HCl dropwise until the bluish tinge just changes to yellow (*p*H 3.0–3.3), avoiding any excess of acid. Add 10 ml 10% KI soln and extract with 20 ml of dithizone soln in CCl_4, 25 mg per liter, shaking vigorously for 2–3 minutes. If extract is red, add more dithizone, and shake again. Allow layers to separate completely and wash down stopper and walls of separator with 3–4 ml of CCl_4, displacing any floating drops of dithizone soln. Draw off the aqueous layer by suction as completely as possible without disturbing the lower layer (about 5 ml remains). Wash down stopper and walls with 25 ml H_2O, but do not shake, and again remove top layer by suction. Add 25 ml 2% KI soln and shake vigorously for 30 seconds. Allow to separate and draw off dithizone

soln into a 125 ml separator, using small portions of CCl_4 to displace floating drops and wash dithizone soln from stopcock bore and stem. Discard KI soln. To the extract add 25 ml 0.01 *N* HCl, and saturated Br water dropwise, with shaking, until dithizone layer is yellow. Shake vigorously 1 minute to transfer Cu to 0.01 *N* HCl. Allow to separate and draw off the CCl_4 layer for recovery. Shake the Cu soln with 10 ml CCl_4 and discard this as before, adding fresh CCl_4 to displace floating drops and the CCl_4 in the stopcock bore and stem. Finally apply a regulated suction of ca 4 inches of water to the top of the separator and admit a stream of air through the lower stopcock until all CCl_4 is aspirated out. If the quantity of Cu is less than 12 micrograms, as evidenced by absence of appreciable color change in the preliminary extraction, use the entire solution for final estimation. If purplish hues develop, draw off the Cu soln into a volumetric flask, wash the separator with 0.01 *N* HCl, add washings to Cu soln, and make to volume with 0.01 *N* HCl.

ESTIMATION OF COPPER

To the 25 ml of soln obtained in "Isolation of Copper," or an aliquot diluted to 25 ml with 0.01 *N* HCl, in the separator, add 5 ml of acid potassium phthalate soln and 1 ml sodium bisulfite soln. From a buret add dithizone soln, 15 mg/l, with intermittent vigorous shaking, until an excess is present as indicated by a purple or grayish-purple color. Add enough CCl_4 to make exactly 15 ml and shake vigorously 1 minute. (If more than 12 ml of dithizone is required, make up to 30 ml with CCl_4 and double the quantity of 0.01 *N* HCl, acid potassium phthalate, and sodium bisulfite. In this case multiply by 2 the quantity of Cu obtained from the calibration chart.) Allow to separate and draw off CCl_4 layer through a pledget of cotton in a funnel into a test tube or small flask. Stopper until readings can be made. Determine optical density at 520 mμ and 625 mμ against CCl_4 as zero, and obtain the amount of Cu from a previously prepared calibration chart or equation.

CALIBRATION

In two or more series of separators, place 25 ml 0.01 *N* HCl containing, respectively, 0, 3, 6, 9, and 12 micrograms of Cu, making 2, or preferably 3, determinations at each level of Cu. Add the acid potassium phthalate and sodium bisulfite solns. To the first separator in each group add a small excess of dithizone, as directed under "Estimation of Copper," and CCl_4 to 15 ml. To the others add increasing amounts of dithizone up to the maximum that can be read accurately in the spectrophotometer. (This is about 8 ml at the zero Cu level and 14 ml at the 12 microgram level.) Extract and determine optical densities at 520 and 625 mμ as directed above. From the results derive the calibration equation, preferably by the Method of Least Squares, in the form:

$$C = AD_{520} - BD_{625} - C_0$$

where

C = concn of Cu in micrograms per 15 ml
D_{520} = density at 520 mμ
D_{625} = density at 625 mμ

and A, B, C_0 are arbitrary constants.

If desired the equation may be charted in nomographic form.

EXPERIMENTAL FINDINGS

Calibration of method. Table 1 shows the data obtained as directed in the method, including the quantities of dithizone found convenient for

TABLE 1.—*Calibration of a spectrophotometer for copper determination by dithizone*

COPPER MICROGRAMS	DITHIZONE SOLN.	CARBON TETRACHLORIDE	DENSITY 520 mμ	DENSITY 625 mμ
	ml	*ml*		
0	3	12	.124	.343
0	6	9	.228	.770
0	9	6	.320	1.07
3	3	12	.211	.180
3	6	9	.320	.560
3	9	6	.430	.910
6	5.5	9.5	.396	.305
6	7	8	.448	.502
6	10	5	.543	.855
9	7.5	7.5	.550	.403
9	12	3	.700	.950
12	10	5	.720	.540
12	15	0	.885	1.11

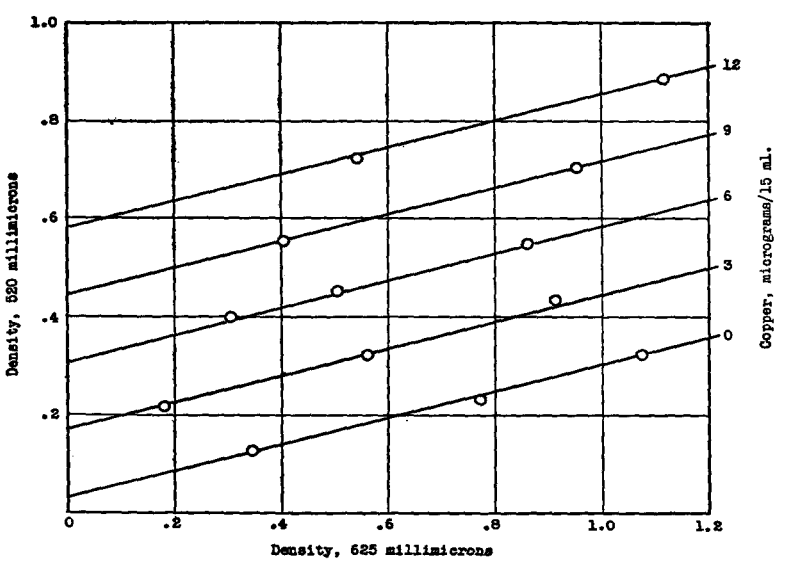

FIG. 1.—Calibration Chart.

covering the useful range of the instrument. The results were best represented by the equation:

$$C = 21.8\ D_{520} - 6.0\ D_{625} - 0.6.$$

Figure 1 shows the results plotted in comparison with this equation, and Figure 2 is a nomograph based on it.

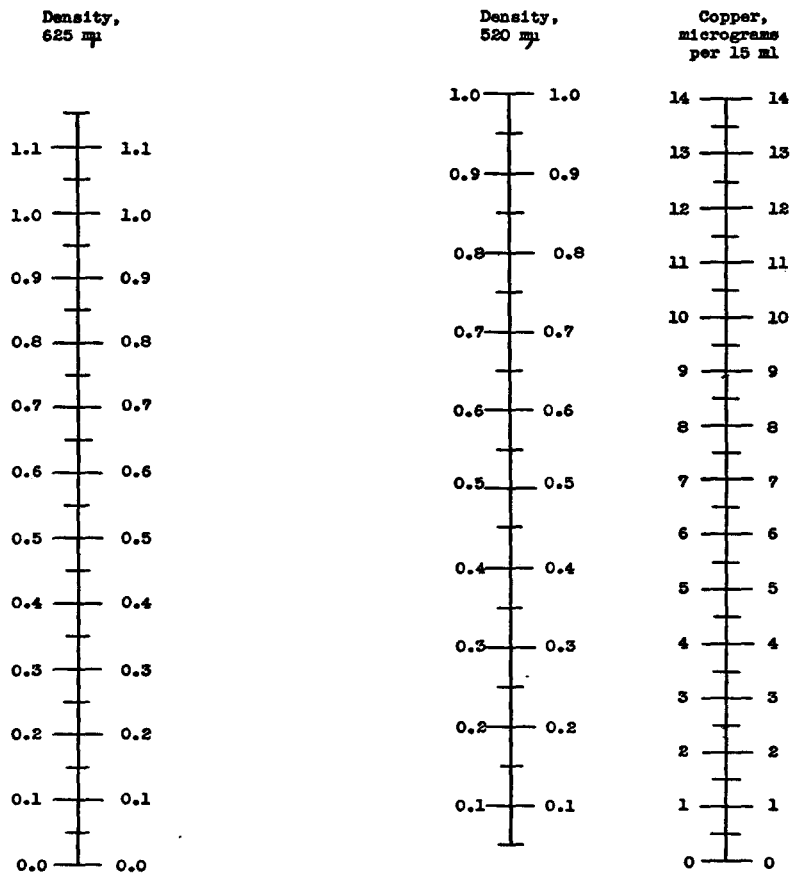

FIG. 2.—Nomograph for Copper Determination.

In attempts to use dithizone solutions which had not been prepared and stored as directed above, erratic results were obtained, evidently due to progressive accumulation of the yellow oxidation product of dithizone, which, like copper, shows higher density at 520 mμ than at 625 mμ. As a

TABLE 2.—*Effect of interfering metals and recovery of copper*

SAMPLE	COPPER ADDED	ZINC ADDED	CADMIUM ADDED	COPPER FOUND	COPPER NET	ERROR
	mmg	*mg*	*mg*	*mmg*	*mmg*	*mmg*
Blank	—	—	—	2.1	—	—
Orange juice, 8 gm.	0	—	—	4.4	2.3	—
	0	—	—	4.4	2.3	—
Orange juice, 8 gm	0	1	1	4.4	2.3	0.0
	0	1	1	4.6	2.5	+0.2
Orange juice, 8 gm	2.5	—	—	7.1	2.7	+0.2
	2.5	—	—	6.9	2.5	0.0
Orange juice, 8 gm	5.0	—	—	9.6	5.2	+0.2
	5.0	—	—	9.5	5.1	+0.1
Orange juice, 8 gm	7.5	—	—	11.9	7.5	0.0
	7.5	—	—	11.9	7.5	0.0
"Spinach," 10 gm (synthetic soln.)	0	—	—	2.3	—	—
	0	—	—	2.2	—	—
"Spinach," 10 gm (synthetic soln.)	2.5	—	—	4.6	2.3	−0.2
	2.5	—	—	4.8	2.5	0.0
"Spinach," 10 gm. (synthetic soln.)	5.0	—	—	7.3	5.0	0.0
	5.0	—	—	7.3	5.0	0.0
"Spinach," 10 gm (synthetic soln.)	7.5	—	—	9.9	7.6	+0.1
	7.5	—	—	9.8	7.5	0.0

result, apparent recoveries of copper, based on earlier calibration with the same dithizone solution, increased as time went on. Evidently, therefore, the purity and stability of the dithizone solution are essential conditions, not only for obtaining maximum accuracy and precision from the method, but for placing dependence on a permanent calibration. Clifford (13) states that CCl_4 dithizone solutions stored in the dark and cold under 0.1 *M* sulfurous acid solution increase in strength for some time and then maintain their strength indefinitely. He recommends that no calibration curves be made until after at least two weeks' storage, to ensure maximum reduction of oxidation products. However, using the method of preparation specified above, it was found that the densities at 520 mμ and 625 mμ did not change appreciably from the initial values during two weeks of storage, and the ratio between the two remained constant within the limits of experimental accuracy.

Elimination of interfering metals.—Aside from bismuth, silver, and mercury, which have been previously shown to be eliminated by the potassium iodide wash, the only metals which might cause concern are zinc and cadmium. When an excess of dithizone is present, zinc extracts in the range *p*H 2.0–5.5, and cadmium from *p*H 2.6–6.1, according to tests made by the author. It was, therefore, anticipated that some zinc, or perhaps both zinc and cadmium, would be extracted along with copper in the preliminary extraction. This was found to be the case, but the amount was unimportant though visible in the first extraction. However, the acidified potassium iodide wash eliminated it entirely, as shown by the results in Table 2.

Recovery of copper.—The remaining figures in Table 2 show the recovery of added copper from two acid digestions, one a digestion of fresh orange juice, the other of a synthetic solution simulating spinach ash. Additions of copper were made at three levels, 2.5, 5.0, and 7.5 micrograms per determination, and recoveries complete to plus or minus 0.2 micrograms were obtained.

SUMMARY

(1) An all-dithizone two-color micro method for copper is proposed. Distinguishing features are the use of variable strengths of dithizone in proportion to the amount of copper in the final determination, and the use of a permanent calibration based on measurement of optical density at two wave lengths.

(2) Preliminary results indicate that the method is free from interferences by other metals, and that it gives good accuracy and precision.

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A QUICK AND SIMPLE METHOD FOR THE DETERMINATION OF QUININE AND TOTAL ALKALOIDS IN CINCHONA BARK

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Quinine is generally considered one of the most important of the *Cinchona* bases since it possesses powerful antimalarial properties. For this reason growers and propagators of *Cinchona* are interested mainly in cultivating and propagating those strains which will produce high yields of quinine. In many instances, such as in an extensive breeding and selection program, it is necessary to analyze a large number of young seedlings for quinine and total alkaloid content. The quantity of sample available for analysis in such cases is generally small (2 grams or less) and the concentration of quinine is usually low (about 1 percent). Most methods for the extraction, separation, and determination of quinine in *Cinchona* bark are tedious, time consuming, and require relatively large samples of 10 to 20 grams. Thus, under certain conditions, these methods are not suitable for obtaining the kind of information desired; a simple rapid method of determining quinine and total alkaloids is needed.

In 1942 and 1943, Carol (2, 3) described a method for the quantitative determination of quinine by absorption spectrophotometry. The method is based on the fact that quinine salts have a strong absorption band in the near ultraviolet with a maximum absorption at approximately 340 mμ. The results obtained from analyses of commercial samples of quinine sulfate by absorption spectrophotometry at 340 mμ were good but, as Carol points out, "Quinine can not be determined by this method in the presence of the other *Cinchona* alkaloids, as they have absorption bands at 340 mμ."

It was felt that if the photometer method could be modified and adapted for use with *Cinchona* bark samples, and if quinine could be determined in the presence of the other *Cinchona* alkaloids, a great saving of time would be achieved. The separation of quinine from the other *Cinchona* bases would thus be obviated and it would be possible to determine quinine directly in the extraction medium.

With this object in view, the present investigation was undertaken. The method as finally developed is as follows:

METHOD

APPARATUS

A spectrophotometer suitable for measuring absorption at 380 mμ. (using the 30 mμ. slit). Matched 1-cm. cuvettes as absorption cells.

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REAGENTS

Ethyl alcohol.—95 per cent distilled.
Calcium oxide.
Hydrochloric acid 0.1 N (concentration not critical).
U.S.P. *XI Quinine sulfate.*

STANDARD SOLUTIONS

Prepare 5 graded standard stock solutions of U.S.P. XI quinine sulfate in 0.1 *N* hydrochloric acid, to contain in five-ml. aliquots 1.6, 3.2, 4.8, 6.4, 8.0 mg. of anhydrous quinine sulfate. This series is equivalent to 2, 4, 6, 8, and 10 per cent, respectively, of quinine sulfate, using a 80 mg. sample of *Cinchona* bark. These solutions will keep 6 months or longer if kept in tightly stoppered black bottles.

DETERMINATION

Extraction.—Weigh two gm. of dried (100 mesh or finer) *Cinchona* bark into a 100 ml. beaker. Add approximately half a gram of finely powdered calcium oxide (the amount is not critical and this can be measured in a spoon) and enough water (7 to 10 ml.) to make a smooth homogeneous paste. After standing for 10 minutes transfer the paste to a 200 ml. volumetric flask with 100 to 150 ml. of ethyl alcohol. Shake the alcoholic bark suspension vigorously and allow it to stand an hour or longer with occasional shaking (about 3 times) after which make it to volume with alcohol, shake and filter thru 24 cm. diameter Whatman No. 5 filter paper. Place a watch-glass over the funnel, and a plug of cotton in the mouth of the Erlenmeyer flask receiving the filtrate, to minimize loss from evaporation of alcohol during filtration.

Determination of quinine.—Pipet 25 ml. of alcoholic extract into a 50 ml. Erlenmeyer flask and add 25 mg. of Norite A, shake for 15–30 seconds, and filter. Pipet 8 ml. of clarified extract (representing 80 mg. of bark) into a 100 ml. volumetric flask, add 5 ml. of 0.1 *N* HCl, make to volume and determine per cent transmittance in photometer at 380 mμ. Read per cent quinine sulfate directly from standard curve prepared as follows:

Pipet 5 ml. of each quinine sulfate stock solution[1] into 100 ml. volumetric flasks. Add 8 ml. of 95 per cent ethyl alcohol, make to volume with water and determine per cent transmittance at 380 mμ in photometer. Use a blank solution containing the acid and alcohol as a reference. Plot curve of log of per cent transmittance vs. concentration. Prepare new curve for each set of determinations.

Total alkaloids.—Pipet one hundred ml. of the alcoholic bark extract (representing 1 gram of bark) into a 400 ml. beaker, add 10 ml. of 0.1 *N* HCl and 100 ml. of water, and titrate the excess acid to *p*H 6.2 with 0.05 *N* NaOH and *p*H meter. Make a blank determination by titrating the alcohol and acid. Subtract the value obtained with the unknown from this blank. One ml. 0.05 *N* NaOH = 0.0155 gram of total alkaloid.

EXPERIMENTAL

Experiments were carried out using dilute solutions of the *Cinchona* alkaloids, mixed and alone. The percent transmittance of light at various wave lengths was measured in a Coleman double monochromator spectrophotometer, Model 105, using the 30 mμ slit in conjunction with a Coleman electrometer, Model 310. It was found that at a wave length of 380 mμ cinchonine and cinchonidine had no effect on the absorption of light. The absorption of light was proportional only to the amount of quinine and quinidine present.

[1] If the range in quinine content of the samples is limited, three standards will suffice.

Solutions containing quinine alone and quinidine alone at the same concentration gave equal transmission values at 380 mμ. Solutions of cinchonine and cinchonidine at different concentrations either alone or combined gave no readings at that wave length. The data presented in Table 1 show that when the method was applied to samples of *Cinchona* bark containing the four *Cinchona* alkaloids in various proportions, the presence of cinchonine and cinchonidine had no measurable effect on the percentage of quinine and quinidine.

Since quinidine resembles quinine in its physiological effects (4), no

TABLE 1.—*Analyses of 10 samples of cinchona bark for four major alkaloids. (A comparison of quinine and quinidine sulfate found by A.O.A.C. methods with that found by photometer method in presence of other alkaloids)*

CINCHONA BARK SAMPLES	CINCHONINE ALKALOID	CINCHONIDINE ALKALOID	QUINIDINE SULFATE	QUININE SULFATE	QUININE + QUINIDINE SULFATE BY A.O.A.C. METHODS	QUININE SULFATE[2] BY PHOTOMETER METHOD
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	1.2	2.1	—	3.8	3.8	3.8
2	—	—	—	7.8	7.8	7.9
3	0.4	0.6	—	8.3	8.3	8.5
4	—	0.5	—	7.0	7.0	7.0
5	—	0.6	0.1	5.1	5.2	4.9
6	1.9	1.3	—	2.1	2.1	2.4
7	1.1	1.1	—	1.4	1.4	1.4
8	—	0.6	0.1	5.0	5.1	5.1
9	0.4	0.6	0.2	3.0	3.2	3.2
10[1]	—	3.0	4.0	—	4.0	4.1

[1] Synthetically prepared sample.
[2] Includes any quinidine present as quinine.

serious error is incurred by its inclusion as an anti-malarial constituent. Also, the quinidine does not occur very frequently, and it is seldom present in large amount, in most *Cinchona* bark (1).

The graph in Figure 1 shows that there was only a small deviation from the Beers-Lambert Law when the log of per cent transmittance at 380 mμ is plotted against concentration of quinine sulfate ranging from 8 to 92 p.p.m.

Tests were made of various technics for extracting the alkaloids from the bark. The one that gave consistent and reproducible results and was simplest to carry out was the one described above.

The data in Table 2 show that immersion of the powdered bark for one hour in ethyl alcohol with occasional shaking (about 3 times) was sufficient to extract the alkaloids quantitatively under the conditions of the test.

One of the main obstacles to using the colored extract as such in the

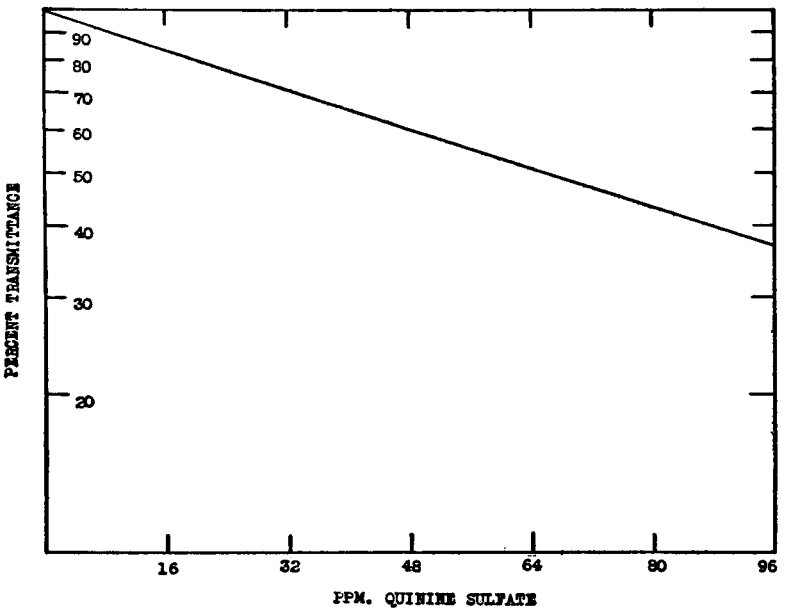

FIG. 1.—Plot of per cent transmittance vs. concentration of graded solutions of quinine sulfate in dilute hydrochloric acid.

photometer was that it gave quinine values 1 to 2 per cent higher than that actually present. Consequently various substances including kaolin, infusorial earth, calcium hypochlorite, and Norite A were tested as decolorizing agents. The best results were obtained with Norite A. It was

TABLE 2.—*Percentage of quinine sulfate obtained in two samples of cinchona bark extracted by immersion of the powdered bark in ethyl alcohol for different periods of time*

TIME OF IMMERSION	SAMPLE 1, (3.8 PER CENT QUININE SULFATE)	SAMPLE 2, (7.0 PER CENT QUININE SULFATE)
hours	*Per cent*	*Per cent*
½	3.7	6.5
1	3.9	7.0
2	3.9	7.0
3	3.8	7.0
4	3.8	7.0
5	3.8	7.0
24	3.8	7.0

found that when 25 ml. of colored alcoholic extract was treated with 25 mg. of Norite A for 15 to 30 seconds and filtered, the color was removed and there was no measurable effect on the quinine content.

Tables 3 and 4 show the effect of treating the colored alcoholic extract with different amounts of Norite A for various lengths of time.

TABLE 3.—*Quinine sulfate in two samples of cinchona bark extract treated with Norite A for various lengths of time. (Twenty-five ml. of alcoholic bark extract treated with 25 mg. Norite A)*

TIME	SAMPLE NO. 2 (2.1 PERCENT QUININE SULFATE)	SAMPLE NO. 3 (8.3 PERCENT QUININE SULFATE)
Seconds	*Per cent*	*Per cent*
15	2.0	8.3
30	2.1	8.2
60	2.1	8.4
120	2.1	8.3

TABLE 4.—*Quinine sulfate in two samples of cinchona bark extract treated with different amounts of Norite A. (Twenty-five ml. of alcoholic bark extract treated for 30 seconds.)*

AMOUNT OF NORITE A	SAMPLE NO. 2 (2.1 PER CENT QUININE SULFATE)	SAMPLE NO. 3 (8.3 PER CENT QUININE SULFATE)
mg	*Per cent*	*Per cent*
25	2.1	8.3
50	1.9	8.0
100	1.2	6.0

It will be seen from Table 3 that 25 mg. of Norite A in 25 ml. of alcoholic bark extract is sufficient to remove the color without any appreciable effect on quinine content, and also that the length of time this amount of Norite is in contact with the solution does not affect the quinine percentage. However, adding more than 25 mg. of Norite to 25 ml. of extract sharply reduces the amount of quinine in the extract (Table 4).

It is apparent from the data presented in Table 5 that fairly good agreement was obtained between samples of *Cinchona* bark analyzed by the official method and by the proposed method. In general the quinine values agreed within 0.1 per cent, with an occasional deviation of 0.2 to 0.3 of one per cent.

Total alkaloids.—Where strict accuracy is required, gravimetric methods are usually preferable to volumetric methods. This is particularly true in the case of the *Cinchona* alkaloids where the molecular weights are high. A small error in titration results in a large error in the amount of alkaloid

TABLE 5.—*Quinine and quinidine sulfate in 15 Cinchona bark samples analyzed by official method and proposed method*

SAMPLE NO.	QUININE SULFATE	QUINIDINE SULFATE	QUININE & QUINIDINE SULFATE BY A.O.A.C. METHOD	QUININE SULFATE[1] BY SPECTROPHOTOMETER	AVERAGE	DIFFERENCE
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	7.8	—	7.8	7.9 7.9	7.9	+.1
2	3.9	—	3.9	3.9 3.9	3.9	.0
3	8.3	—	8.3	8.6 8.4	8.5	+.2
4	4.2	—	4.2	4.3 4.3	4.3	+.1
5	7.0	—	7.0	6.9 7.1	7.0	−.1
6	3.5	—	3.5	3.5 3.5	3.5	.0
7	5.0	0.1	5.1	5.0 4.9	5.0	.0
8	4.0	—	4.0	3.8 3.9	3.8	−.2
9	3.4	—	3.4	3.4 3.4	3.4	.0
10	4.2	—	4.2	4.2 4.2	4.2	.0
11	2.4	—	2.4	2.5 2.5	2.5	+.1
12	5.0	—	5.0	5.1 5.0	5.1	+.1
13	1.9	0.2	2.1	2.4 2.4	2.4	+.3
14	1.4	—	1.4	1.5 1.4	1.4	+.0
15	3.8	—	3.8	3.8 3.8	3.8	−.0

[1] Includes any quinidine sulfate present.

found. However, where large numbers of samples are involved and highly accurate results are not required, a great saving of time may be effected by the titration method.

Accordingly, tests were conducted with aliquots of the alcoholic bark extract which included evaporating off the alcohol and dissolving the residue in various amounts of standard acids, filtering and titrating the excess acid with standard NaOH, using different indicators. The simplest and most successful method for titrating the *Cinchona* alkaloids was that described on page 154.

In Table 6 data are presented which show the comparison of total alkaloid content of 10 *Cinchona* bark samples analyzed gravimetrically and by titration of alcoholic extract with *p*H meter. The values obtained by titration are generally somewhat higher than those obtained gravimetrically. However, a good approximation of the total alkaloids in *Cinchona* bark can be obtained by the titration method and it has the advantage of being rapid and requiring relatively small samples.

TABLE 6.—*Comparison of per cent total alkaloids found in 10 samples of cinchona bark by gravimetric method and by titration method*

CINCHONA BARK SAMPLES	TOTAL ALKALOID (GRAVIMETRIC METHOD)	TOTAL ALKALOID (TITRATION OF ALCOHOLIC EXTRACT USING *p*H METER)
No.	*Per cent*	*Per cent*
1	6.0	5.8
2	7.0	7.4
3	6.6	6.8
4	5.1	5.2
5	5.2	5.5
6	7.0	7.1
7	6.1	6.2
8	5.0	5.2
9	8.0	8.2
10	6.4	6.7

SUMMARY

A simple rapid method of extracting and determining quinine and total alkaloids in *Cinchona* bark is described.

The method has the advantage of requiring small samples of bark (1 and 2 grams) and it can be used to determine small amounts of quinine; any quinidine present is also measured as quinine. Thus, the method may be useful in evaluating *Cinchona* seedlings for anti-malarial constituents at an early stage in their life cycle. With a rapid simple method for assaying cinchona bark, large numbers of seedlings can be "screened" in a relatively short time, thus facilitating such projects as a breeding and selection program. The method can also be used to assay samples of *Cinchona* bark for commercial purposes.

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(4) Merck Index. Fifth Edition. 1940. Published by Merck & Co., Inc., Rahway, N. J., p. 468.

PROPOSAL FOR MODIFICATION OF THE WAGNER PROCEDURE AND ITS ADAPTATION FOR P_2O_5 "AVAILABILITY" OF FUSED TERTIARY PHOSPHATES*

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The present official procedure (1) for the determination of citric acid soluble phosphoric acid in basic slag is virtually identical to the one proposed by Wagner in Germany some 60 years ago. Objection has been made to his stipulation for so large an analytical charge and to the attendant wastage of so large a quantity of an expensive reagent. Moreover, the prescribed flask is unusual and its size necessitates cumbersome agitation apparatus. Consequently, some laboratories have assumed that rational deviations from the original technique do not alter the principle of the Wagner procedure and, hence, have applied such deviations in the analytical evaluation of *basic slag*.

Producers of fused phosphatic fertilizers, and control officials in two States, have asked for advice as to the appropriate procedure for the chemical evaluation of fused tricalcium phosphate and fused tricalcium-magnesium phosphate. Since the principle of the Wagner method is deemed well adapted to the determination of the "available" P_2O_5 content of slags, it seemed logical to utilize that procedure for the evaluation of new thermally processed phosphatic materials. The prescribed 2 per cent citric acid solvent is adequately acidic to effect the dissolution of the several types of unacidulated fertilizer phosphates without sequential reagent neutrality or alkalinity that would diminish dissolvent effectiveness.

It might be contended that new fused phosphatic fertilizers are subject to the classification of "non-acidulated" products "other than basic slag" (1). The procedure prescribed for such "other than" products stipulates "without previous washing" and thus is variant from the one used for "acidulated samples." But, since the procedure prescribed for the "other than" products was incorporated as an official method years before the advent of fused phosphatic fertilizers, it is obvious that these phosphates were not considered in connection with the cited procedure. This Association does not prescribe a procedure until the fertilizer has been officially defined.

It was because of such lack that the fused tricalcium phosphates from electric furnaces were subjected to the earlier studies in which P_2O_5 availability was determined by means of neutral ammonium citrate and 2 per cent citric acid, under various conditions (2). The P_2O_5 values registered

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† The present study was conducted in the Cooperative Chemical Research Unit of the Department of Chemical Engineering, Tennessee Valley Authority, at the Agricultural Experiment Station of the University of Tennessee.

by the acidic solvent were considerably higher than those indicated by the neutral ammonium citrate solution, and were also more consonant with the values indicated by plant response through integrated pot culture studies. This consonance was substantiated by subsequent comparisons in which the citric acid dissolvable P_2O_5 content of fused tricalcium phosphate from the blast furnace proved as effective an index as the available P_2O_5 content of superphosphate.

EXPERIMENTAL

The present official procedure prescribes a 5-gram charge of basic slag weighed into a 500 ml. cylindrical flask (Wagner) containing 5 ml. of alcohol, addition of 2 per cent citric acid to volume, and 30 minutes agitation by means of an end-over-end shaker at 30–40 r.p.m. The principle of the Wagner procedure has been retained in each of the following modifications in technique for 100-mesh material:

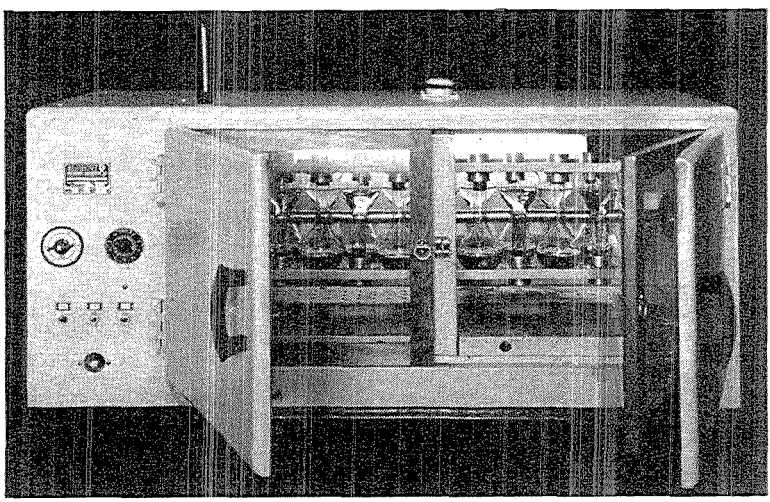


FIG. 1.—Device* for end-over-end agitation used for Modification I, Tables 2 and 3.

Modification I.—Into dry 250-ml. "fertilizer" flask weigh 1-gram charge and, while rotating the flask to prevent caking, deliver 100 ml. of 2 per cent citric acid solution by means of pipet or dispensing buret. Stopper flask, insert into the end-over-end shaking machine† (Fig. 1) and agitate continuously at 20–30 r.p.m. for 30 minutes at room temperature. Remove flask, dilute contents to mark with distilled H_2O, mix thoroughly and filter immediately through either a dry fluted filter or a Shimer filter under suction. Discard the initial 25-ml. portion of the filtrate,

* Developed from the homemade original, illustrated and sketched in *This Journal*, May 1944. The cabinet is not needed when the end-over-end agitations are at room temperature, as in Modification I.

† Or the homemade device (3), minus cabinet for temperature control.

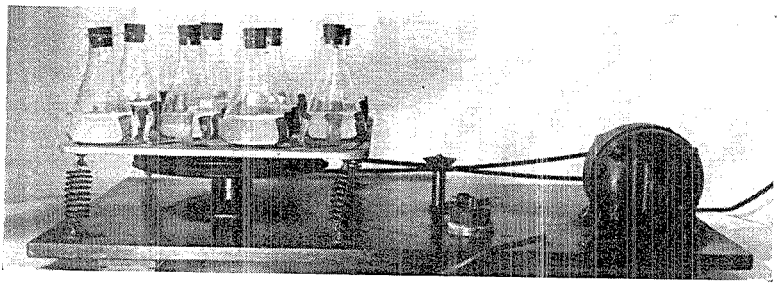


FIG. 2.—Ross-Kershaw "shaker" used for Modification II of Tables 2 and 3.

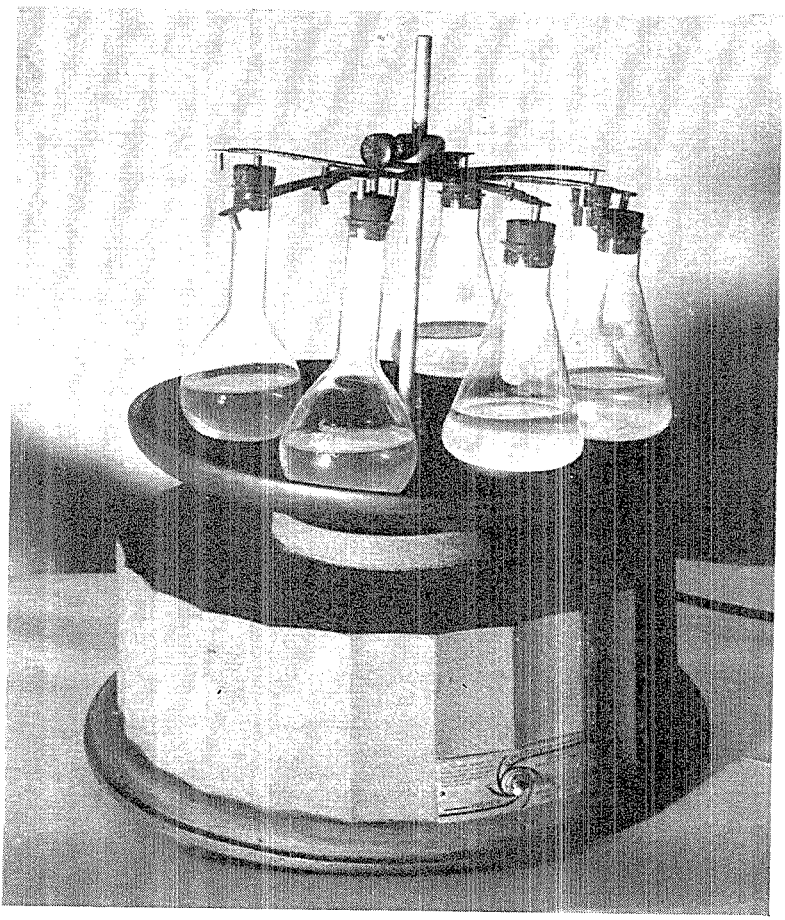
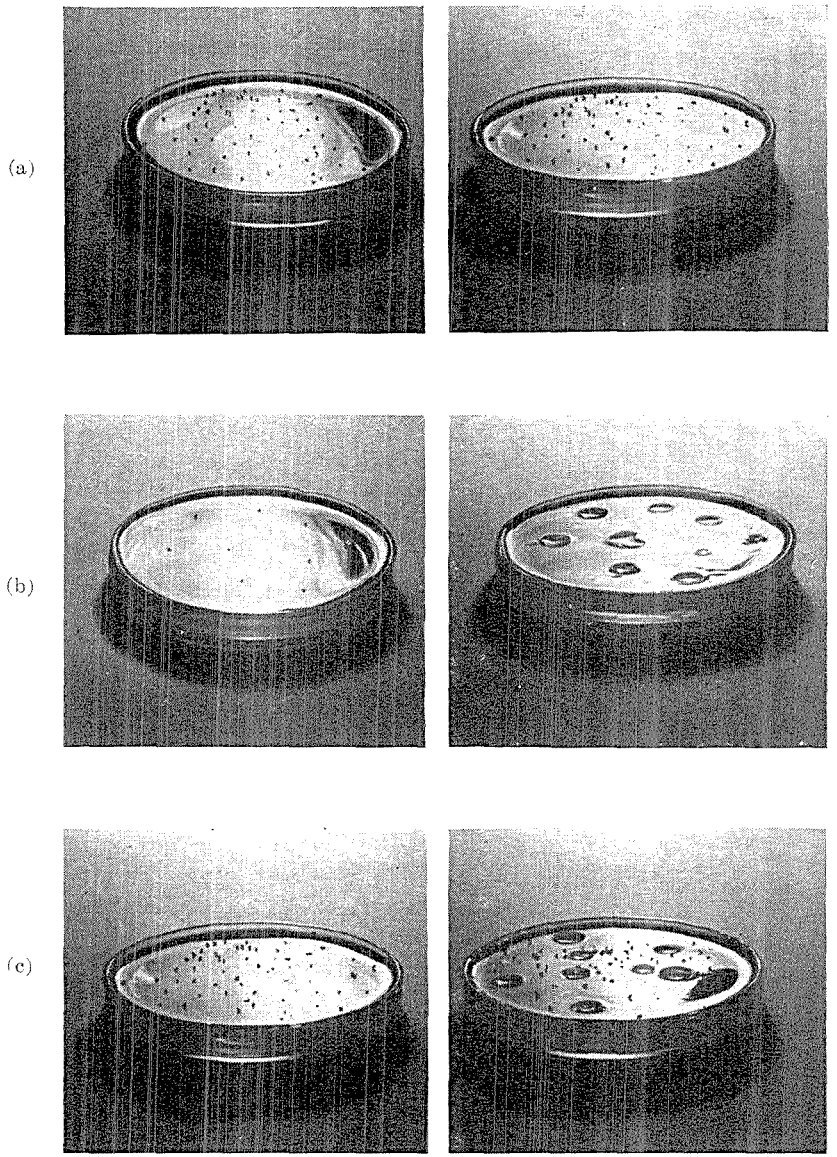


FIG. 3.—"Gyrosolver" used in Modifications III and IV, Tables 2 and 3.



Before incubation After incubation

FIG. 1.—Effect of sage and excreta on gelatin plates before and after incubation at 25°C.

(a) sage; (b) excreta; (c) mixture of sage and excreta.

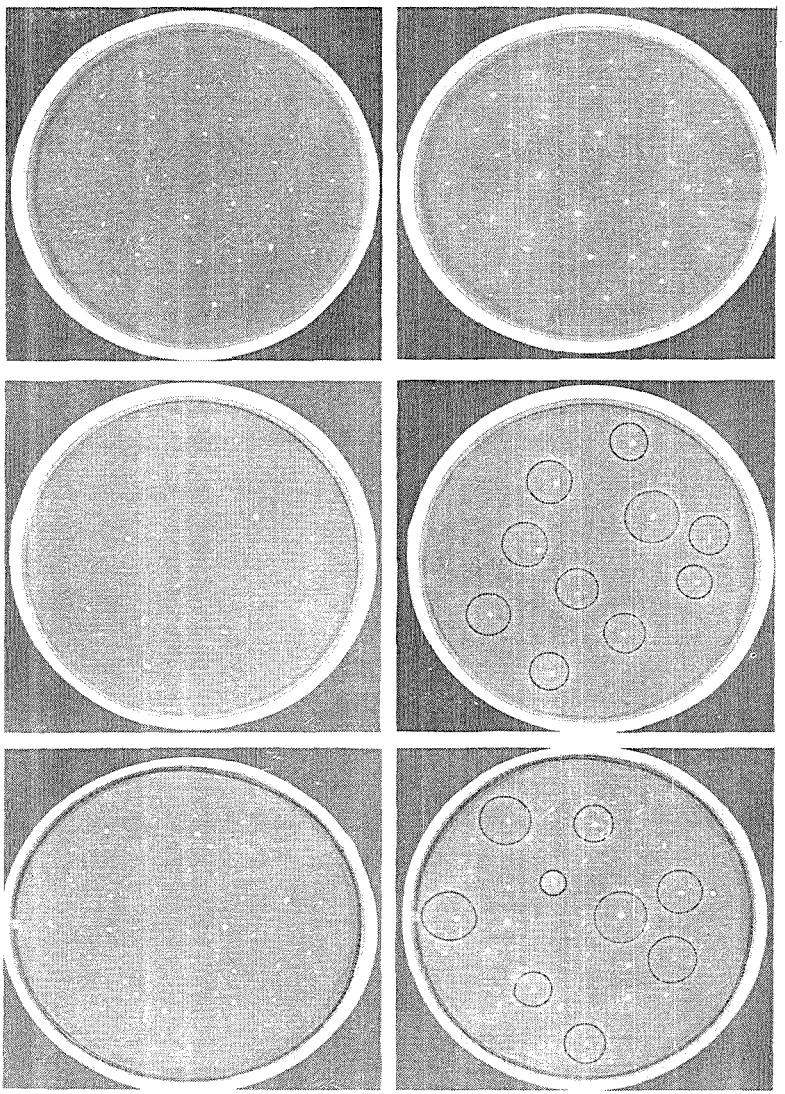
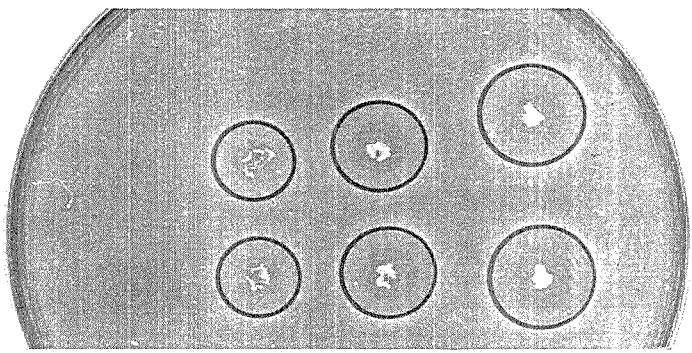


FIG. 2.—Photographic records of the effect of sage and excreta on gelatin plates before and after incubation at 25°C.

(a) sage; (b) excreta; (c) mixture of sage and excreta

ficient protease to give positive tests by the gelatin plate method. However, seeds on which no smut could be seen under the microscope gave no proteolytic action. It has also been noted that, in some stages of growth, other fungi are active. Since there is action from fungi, it is desirable to differentiate between the action from excreta and fungi. It is also desirable to differentiate insect from rodent excreta action.

The gelatin plate technique appears susceptible to considerable development. Some study was devoted to varying conditions and to applying activators and inhibitors, with the hope of rendering the procedure specific



(a) (b) (c) (d)

FIG. 3.—Circles obtained from different concentrations of trypsin in .01 ml droplets.

(a) 0.0 mg trypsin powder (Pfanstiehl 1–110) in .01 ml.
(b) 0.05 mg trypsin powder (Pfanstiehl 1–110) in .01 ml.
(c) 0.10 mg trypsin powder (Pfanstiehl 1–110) in .01 ml.
(d) 0.20 mg trypsin powder (Pfanstiehl 1–110) in .01 ml.

for pancreatic trypsin. Means of promoting its quantitative aspect were also considered. It was possible to differentiate pepsin from trypsin by control of *p*H, and to differentiate the plant protease, papain, from trypsin by addition of an oxidizing agent such as potassium iodate to the gelatin, in which case papain action was completely arrested and trypsin action was uninhibited. The protease of *Aspergillus Oryzae* behaved similarly to trypsin with respect to *p*H and was not affected by oxidizing or reducing agents to an extent that was measurable by this technique. The gelatin plates are, however, of value as a sorting test. If contamination is indicated thereby, activity due to fungi could probably be confirmed or ruled out by a microscopic examination of the active particles. In case sufficient activity due to fecal contamination alone were indicated by these tests, a quantitative estimate could then be made.

Attention was then directed to study of a quantitative procedure which

even though less delicate for detection of positive action from extremely small fecal particles might lead to conclusions applicable to the gelatin plates. Anson's hemoglobin method (1) appeared to be the most convenient and delicate quantitative method. In this method hemoglobin is hydrolyzed by a protease under standard conditions of time, temperature, and *p*H. The liberated substances, not precipitable by trichloracetic acid, which reduce Folin's phenol reagent (tungstate-molybdate reagent) are estimated colorimetrically. An attempt was made to use the method unmodified on extracts of contaminated food products, but the extracts contained other substances which reduced the tungstate-molybdate reagent and thus prohibited its use. A modification was therefore introduced by using the phenol reagent of Hanke and Koessler (diazotized sulfanilic acid) (13), which in many instances gave much lower blanks.

The color developed by the Hanke-Koessler procedure does not compare to the color developed from any simple product of the digestion, and no single organic compound was found that could be used as a standard. A mixture of cobalt chloride and sodium dichromate gave a color which was sufficiently close in appearance, and in spectral characteristics in the region of 460 mμ wave length, to provide a satisfactory reference standard when a neutral wedge photometer was used.

As yet we have not been able to use the quantitative method for the determination of small amounts of trypsin in food extracts (5 micrograms of Pfanstiehl 1:110 trypsin powder or 0.1 to 0.2 milligrams of excreta in one gram of food product) because the extracts contain inhibitors which (as in the case of corn, bean, and pea meals) completely stop the action from a small amount of trypsin, or else they contain sufficient plant enzyme to give appreciable proteolytic action (as in the case of bean and pea meals). None of these three difficulties are encountered with the gelatin plates. Means of avoiding these difficulties and thus adapting this technique to the estimation of the degree of fecal contamination are still under investigation.

(1) GELATIN PLATE METHOD FOR FECAL DEMONSTRATION OF PROTEOLYTIC ACTION

Gelatin.—Keystone Silver Label or equivalent.

Buffer antiseptic soln.—Dissolve 2.866 gm $Na_2B_4O_7 \cdot H_2O$, 4.341 gm H_3BO_3, and 0.5 gm ortho-cresol in water and dilute to 500 ml in volumetric flask.

Preparation of gel.—Dissolve 15 gm gelatin (cut into small pieces) in 100 ml of buffer antiseptic soln at about 50°C., accompanied by gentle stirring. Pour into separatory funnel and allow bubbles to rise a few minutes. Open stopcock and with tip of funnel resting on bottom of petri dish, allow gelatin to flow slowly into petri dish until surface of gelatin reaches a height of 5/16 inch above level surface on which plate is setting. A depth gauge supported by two 1-inch blocks is convenient for obtaining proper height of gelatin. Allow to gel.

Distribution of sample on gelatin plate.—Hold a sieve of the appropriate sieve size (depending on the size of particles involved), containing the ground material, over the gelatin plate. Tap the sieve gently to distribute the particles evenly over the surface of the gelatin. Endeavor to distribute the material in a single layer in

order to prevent active particles from being held off the surface of the gelatin by inactive particles.

(2) HEMOGLOBIN METHOD[1] FOR TRYPSIN IN FECES

REAGENTS

Preparation of hemoglobin soln.—Defibrinate freshly drawn beef blood by whipping, immediately after collection, with a well-tinned wire beater (avoid iron utensils). Throw the fibrin off the beater as it collects and continue the beating for ca 15 min. Centrifuge the defibrinated blood 20–30 min. and siphon off the serum and thin layer of white corpuscles. Mix the red corpuscles with an equal volume of cold 1% sodium chloride soln, centrifuge, and siphon off the supernatant liquid. Put the corpuscles and one marble in each of several cellophane tubes and dialyze for 24 hours against cold running tap water. Run the water into the bottom of the container at a sufficient rate to cause stirring, and occasionally tilt the cellophane tubes back and forth to allow the marbles to stir the hemoglobin solns. Following the dialysis mix together the hemoglobin solns from all the tubes. To estimate the protein concentration weigh a 3–5 gm sample into a porcelain evaporating dish, dry overnight at 105°C., and weigh. Calculate as follows:

$$\text{No. of gm of hemoglobin (Hb) per ml of sample} = \frac{\text{wt of dried Hb}}{(\text{wt of sample} - \text{wt of dried Hb}) + 0.73 \text{ wt of dried Hb}}$$

Dilute the soln to 22.0 gm per 100 ml and store frozen in small aluminum or waxed cardboard containers.

Sodium hydroxide soln.—1 *N*.

Hemoglobin substrate soln.—Prepare a soln containing 8 ml of 1 *N* sodium hydroxide, 72 ml water, 36 gm urea, and 10 ml of 22% hemoglobin soln. Keep this alkaline soln at 25°C. for 30–60 min. to denature the hemoglobin. Then mix it with a soln containing 10 ml of 1 *M* potassium dihydrogen phosphate and 4 gm of urea. Adjust with sodium hydroxide or phosphoric acid if the *p*H is not exactly 7.5. Add 1 mg merthiolate (Lilly) to each 50 ml of hemoglobin substrate soln and store at 5°C.

Trichloracetic acid soln.—0.3 *N* (estimate by titration).

Stock sulfanilic acid soln.—Dissolve 3.374 gm of sulfanilic acid in about 300 ml of water and 33.7 ml of 36% hydrochloric acid, and dilute to 500 ml in a volumetric flask. The sulfanilic acid dissolves slowly but completely.

Stock sodium nitrite soln.—5 per cent. Store in refrigerator at 5°C.

Sodium carbonate soln.—Dissolve 5.50 gm of anhydrous sodium carbonate in water and dilute to 500 ml in a volumetric flask. Store in a Pyrex bottle.

Sodium hydroxide soln.—2 *N*.

Hydroxylamine hydrochloride soln.—20 per cent.

Color standard soln.—Dissolve 20.000 gm of $CoCl_2 \cdot 6H_2O$ and 0.4125 gm of $Na_2Cr_2O_7 \cdot 2H_2O$ in 1% hydrochloric acid soln (1+99), and dilute to 1 liter in a volumetric flask with the 1% hydrochloric acid soln.

Color reagent (p-diazobenzenesulfonic acid).—Mix 2.0 ml of stock sulfanilic acid soln and 1.5 ml of stock sodium nitrite soln in a 50 ml volumetric flask. Immerse flask in ice bath for 5 min., add 6.0 ml stock sodium nitrite soln, mix and immerse in the ice bath for an additional 5 min. Dilute to 50 ml with distilled water, mix and keep in ice bath. Do not use reagent for 15 min. Prepare fresh daily.

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[1] This hemoglobin method was used to determine the proteolytic value of the excreta samples which are presented in this paper.

PROCEDURE

Preparation of fecal suspension (enzyme soln).—Powder fecal material with mortar and pestle. Triturate 25 mg of this fecal powder in distilled water and dilute to 50 ml in volumetric flask. Mix well immediately before withdrawing aliquot.

Digestion.—Dispense 5.0 ml of hemoglobin substrate soln into a glass-stoppered test tube and place in a constant temperature water bath at 37°C. Bring the enzyme soln to the same temperature and then add 1 ml, or a definite fraction thereof, to the hemoglobin substrate. Swirl the tube to mix the contents without splashing the soln onto the ground glass joint and replace the tube in the bath for an incubation period of 4 hours. After incubation add 10 ml of 0.3 *N* trichloracetic acid soln, shake the tube vigorously and allow to stand for 30 min. Filter through Whatman No. 3 paper or equivalent and retain filtrate. Make a blank determination in exactly the same manner as the unknown determination, only add the enzyme soln after the trichloracetic acid precipitation instead of before the incubation period.

Color development.—Measure 5 ml of 1.1% sodium carbonate soln into a suitable tube (a 25 ml cylinder is convenient). Add 2 ml of color reagent to the alkali with a 5-second delivery pipet, note the exact time, and mix the contents by swirling the tube. At the end of exactly 1 min. add 1 ml of the digestion filtrate using a fast delivery pipet, and again mix by swirling. Exactly 2 min. after the addition of the digestion filtrate, add 2 ml of 2 *N* sodium hydroxide, mix, and allow to stand 1 min. Then add 2 drops of 20% hydroxylamine hydrochloride soln, mix the tube, and determine the color intensity within 2 hours by means of the neutral wedge photometer (8), using 1 inch cell and No. 46 filter. It is convenient, using one stop watch, to develop the color in two tubes at one time by staggering all steps one-half min. apart.

Color standard and reference curve.—Use 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 ml, respectively, of the standard color soln and dilute each portion to 100 ml with 1% hydrochloric acid. Read in photometer using 1 inch cell and No. 46 filter. Make a graph of the standards, plotting ml of standard color soln against photometer readings corrected for 0 ml reading. By means of this plot, convert photometer reading obtained from the 4 hour digestion mixture (corrected for blank) to ml of standard color (which are arbitrarily called units). It is necessary to make only one standard curve, provided the adjustment of the instrument is not altered.

PHOSPHATASE

Phosphatases hydrolyze $PO_4\equiv$ from phosphoric acid esters. Some mammalian feces contain a relatively high concentration of alkaline phosphomonoesterase (intestinal phosphatase), whereas mold, insect excreta, and plant materials are reported to contain phosphatases having a much lower optimum *p*H (19, 9).

Intestinal phosphatase is reported to react differently than some other phosphatases to such reagents as cyanide, fluoride, oxalate, and magnesium ions (21,17). Therefore, the determination of the alkaline phosphatase activity of contaminated food products seems promising for differentiating rodent excreta from insect excreta and mold contamination. It has been observed that particles of rodent and insect excreta with about equal gelatin liquefying power show a great difference in phosphatase activity. If these particles are placed in drops of a buffered alkaline phenolphthalein phosphate solution (14), the drop containing the rodent excreta turns red

on incubation and the drop containing the insect excreta remains colorless. The same difference can be demonstrated on a solidified agar plate containing phenolphthalein phosphate,[2] which is similar to the bacteriological technique of Bray and King (7). Large fragments of rodent excreta are required to produce a red color when gelatin plates containing phenolphthalein phosphate are used. Moreover, the color is faint and gradually fades on standing. Smaller fragments of excreta, which give definite proteolytic action, give no red color in the gelatin plates. It has not been determined whether the gelatin and the products of hydrolysis inhibit the phosphatolytic action, or whether they otherwise interfere with the color development. Disodium p-nitrophenyl phosphate (6) has not been tried.

Phosphatase activity of excreta can be estimated quantitatively by determining the amount of $PO_4\equiv$ liberated from sodium glycerophosphate under standard conditions of *p*H, time, and temperature. If $PO_4\equiv$ normally present causes high blanks, it can be removed by dialysis before the solution is tested for phosphatase activity. The usual procedures for the quantitative determination of the liberated $PO_4\equiv$ (the formation of phosphomolybdic acid and the subsequent blue color formation by the addition of a suitable reducing agent) were unsatisfactory. The aqueous extracts contained substances precipitable by the molybdic acid reagent, resulting in turbidity in the final blue color. Filtration of the turbid blue solution caused loss in color intensity, and the use of more trichloracetic acid before color development, to avoid the turbidity, caused erratic results. However, the procedure of Pons and Guthrie (20) in which the phosphomolybdic acid is partitioned into isobutyl alcohol before addition of the reducing agent gave reproducible and very satisfactory results. This procedure was used for the analyses presented in this paper.

In aqueous extracts made from some contaminated food samples the amount of acid phosphatase, from the relatively larger amount of plant material, is much greater than the amount of alkaline phosphatase from the excreta. This results in overlapping of *p*H activity curves to such an extent that the alkaline phosphatase cannot be measured quantitatively. However, adsorption and purification of the different phosphatases (and proteinases) from contaminated samples are under investigation.

(3) METHOD FOR PHOSPHATASE IN FECES

REAGENTS

Buffer-substrate soln.—Dissolve 2.473 gm sodium barbital and 1.55 gm sodium glycerophosphate $\cdot 5H_2O$ (Eastman Kodak) in approximately 50 ml water. Add 5.0 ml 0.1 *N* HCl and dilute to 100 ml in a volumetric flask.

Magnesium chloride soln.—Dissolve 0.020 gm $MgCl_2 \cdot 6H_2O$ in 100 ml water in a volumetric flask.

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[2] Since the preparation of this manuscript the article "A Micro-method for the Detection of Phosphatases by 'Agar-plate' Method" by Das *et al*, *Science and Culture*, 11, 700 (1946), has been brought to our attention.

Trichloracetic acid soln.—Dissolve 37 gm trichloracetic acid in 100 ml water.

Molybdate reagent.—Dissolve 50 gm ammonium molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) in 400 ml 10 *N* sulfuric acid and 500 ml of water, make up to 1 liter in a volumetric flask, and store in a paraffin-lined bottle.

Sulfuric acid, approximately N.—Dilute 114 ml of concentrated sulfuric acid to 4 liters.

Stannous chloride stock soln.—Dissolve 10 gm stannous chloride in 25 ml concentrated hydrochloric acid. Store in a small, glass-stoppered, brown bottle.

Stannous chloride dilute soln.—Dilute 1 ml stock soln to 200 ml with approximately *N* sulfuric acid. Prepare just before use.

Isobutyl alcohol.—Commercial grade, with a boiling range 106° to 110°C., is satisfactory.

Ethyl alcohol.—95 per cent.

Standard phosphate soln.—Recrystallize A.C.S. grade monobasic potassium phosphate three times with water, dry at 110°C., and store in a desiccator over concentrated sulfuric acid. Dissolve 4.3929 gm of dry salt in 300 ml of water and 200 ml approximately *N* sulfuric acid. Add a few drops of 0.1 *N* potassium permanganate as a preservative and make up to 1 liter in volumetric flask with water. This stock solution, 1.0 mg of phosphorus per ml, is stable. Dilutions are made as needed.

PROCEDURE

Preparation of fecal suspension (enzyme soln).—Prepare as directed under hemoglobin method.

Digestion.—Measure 4.0 ml of buffer-substrate soln, 1.0 ml magnesium chloride and 4.0 ml distilled water into a glass-stoppered test tube and warm to 37°C. Add 1.0 ml of enzyme soln (fecal suspension) warmed to 37°, mix by swirling, and allow to incubate at 37°C. After one hour, add 5.0 ml trichloracetic acid and filter on quantitative paper of medium retentivity. Make a blank determination in exactly the same manner as the unknown determination except add the enzyme soln after the trichloracetic acid precipitation instead of before the incubation period.

Phosphorus determination.—Pipet an aliquot of digest filtrate containing from 5 to 45 micrograms of inorganic phosphorus and a like aliquot of blank filtrate into separate 125 ml separatory funnels with a mark at 20 ml. Add 5 ml of the molybdate-sulfuric acid reagent, and distilled water to the 20 ml mark. Add 10.0 ml of isobutyl alcohol and shake for 2 min. Discard the aqueous layer and wash by shaking once with 10 ml of approximately *N* sulfuric acid. Add 15 ml of dilute stannous chloride soln, shake for 1 min., allow to separate, then discard aqueous layer. Transfer the blue isobutyl alcohol layer to a 25 ml volumetric flask, wash the funnel with ethyl alcohol and add washings to volumetric flask. Make to volume with ethyl alcohol. Determine the color intensity by means of the neutral wedge photometer (8), using 2-inch cell and No. 65 filter, at any time from 40 min. to 4 hours after color development.

Phosphorus standard.—Prepare a standard curve by pipetting known concentrations of inorganic phosphorus in the range 0 to 45 micrograms (obtained by diluting stock phosphate soln) into 125 ml separatory funnels and developing the color exactly as outlined in the procedure. Make a graph of the standards, plotting phosphorus against photometer readings corrected for 0 phosphorus reading. By means of this plot, convert photometer reading of the unknown (corrected for blank) to micrograms of phosphorus. It is necessary to calibrate the photometer but once, provided the adjustment is not altered, since the standard curve has been found to be reproducible.

SAMPLES AND ANALYSES

Filth in food products obviously does not need to be determined with high precision. Yet to demonstrate that more than an infinitesimal fecal contamination is present necessitates that the method employed be at least roughly quantitative. With this objective the potency of 33 rodent, and 8 insect, excreta samples was determined. These samples were collected from commercial food processing plants or from exhibits of contaminated food products. The excreta pellets were powdered with a small mortar and pestle. Solutions were prepared for analysis by triturating 25 mg of the powder in distilled water and making the suspension to 50 ml. Aliquots (usually 1 ml) of these suspensions were used as the enzyme preparations in the foregoing hemoglobin protease method and in the phosphatase method. Results are shown in Table 1.

DISCUSSION

Certain enzymes show promise of being specific indicators of the presence of excreta. When the gelatin plates are used, the presence of one very minute particle of high proteolytic potency can be detected. Since we have so far found excreta and fungi to be the only contaminants in normal commercial food products which have sufficient potency to give tests by the gelatin plate method, it is reasonable to assume that this test is specific. Although the difference in action between fungi and rodent excreta has not as yet been demonstrated, there are indications that such a differentiation can be made by enzyme technique. Investigations in this direction are now under way.

Table 1 shows that all of the insect excreta samples so far encountered have had very low alkaline phosphatase activity. This appears to be a good basis for discriminating between rodent excreta or fungi, and insect excreta.

The values shown in the table cover a fairly wide range but they still make it possible to employ the enzyme content as a useful index of the degree of contamination. These samples do not necessarily represent normal excreta values since their history is not known, but they do indicate the values that may be found in samples from food products. The extreme range for the protease is about 20 fold, but the phosphatase range is greater. The gelatin plate is immediately useful as a qualitative indicator of fecal contamination, even though it is at present impracticable to employ the plate quantitatively. (There appears to be some possibilities in this direction, however.) Furthermore, the qualitative value of gelatin plates may be supplemented by the application of other quantitative procedures in order to establish whether or not the degree of contamination is substantial. These quantitative procedures are not simple, but the promise of their usefulness seems to justify continuing their development.

TABLE 1.—*Trypsin and alkaline phosphatase values of excreta*

SAMPLE NUMBER	ANIMAL	TYPE OF FOOD OR FACTORY WHERE FOUND	TRYPSIN VALUE (UNITS PER MG)	ALKALINE PHOSPHATASE (MICROGRAMS LIBERATED PER MG IN 1 HR.)
1	Mouse	Fed bird seed	30	41
4	Mouse	Rye flour	24	40
10	Mouse	Macaroni factory	14	15
28	Mouse	Mustard seed	36	150
29	Mouse	Brewers' flakes	28	28
30	Mouse	Brewers' flakes	31	38
33	Mouse	Flour	111	365
34	Mouse	Flour	142	103
35	Mouse	Flour	15	87
36	Mouse	Flour	106	243
37	Mouse	Flour	128	480
	Average		60	145
3	Rat	Coffee beans	20	21
4a	Rat	Bran	21	60
5	Rat	Walnuts	44	86
6	Rat	Coriander seed	16	45
6a	Rat	Coriander seed	7	60
6b	Rat	Chili peppers	8	71
7	Rat	Cacao nibs	62	184
7a	Rat	Cacao nibs	21	47
7b	Rat	Cacao nibs	68	158
9	Rat	Macaroni factory	23	15
9a	Rat	Macaroni factory	23	17
9b	Rat	Macaroni factory	22	21
9c	Rat	Macaroni factory	30	47
11	Rat	Dried prunes	55	37
11a	Rat	Dried prunes	74	19
24	Rat	Flour	14	8
25	Rat	Flour	15	17
26	Rat	Flour	16	61
31	Rat	Brewers' flakes	20	31
32	Rat	Brewers' flakes	13	58
38	Rat	Brewers' flakes	14	113
	Average		28	56
8	Rat & mouse	Flour	92	113
12	Larvae & beetles	Dried prunes	31	2
14	Unident. insect	Peanuts	135	0
15	Grainery beetles	Pearl barley	9	2
19	Unident. insect	Cacao	33	2
20	Unident. insect	Cacao	81	1
21	Unident. insect	Dried beans	113	0
22	Unident. insect		178	2
23	Cockroach		15	3
16	Chicken		91	4
17	Rabbit		7	0
18	Guinea pig		8	3
27	Aspergillus oryzae	Grown on bran	6	3

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THE POLAROGRAPHIC DETERMINATION OF ZINC IN SOIL*

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The discovery of soils which are deficient in available zinc for the normal growth of plants has developed a need for a reliable and accurate method for the determination of zinc in soil. Sherman and McHargue (3) described a colorimetric method in which the soil sample is either digested with a combination of perchloric and hydrofluoric acids or fused with mixed alkali carbonates to bring the zinc into solution. The zinc is determined by measuring the light transmission through a dilute solution of zinc dithizonate. A similar procedure has been described by Holmes (1).

Walkley (4) and Piper (2) have found a polarographic method to be the most convenient one for determining the small amounts of zinc which occur in plant tissue. In their procedure the zinc is separated from the bulk of inorganic constituents by extraction with a chloroform solution of dithizone at *p*H 9.8. The organic matter is destroyed by wet digestion and the acid completely volatilized by evaporation to dryness. The residue is dissolved in a measured volume of a supporting solution containing ammonium chloride and potassium thiocyanate, and polarized between potentials of 0.8 and 1.2 volts at the dropping mercury electrode. The optimum amount of zinc for this determination was about 3–300 micrograms, and in this range they were able to obtain an accuracy of ± 2 per cent.

The polarographic method for the determination of zinc has been found to be applicable to a large number of soil samples in this laboratory. The procedure as adapted to soils has proven to be accurate and rapid. Because of its accuracy over a wide range of zinc concentration the method is recommended to soil analysts.

POLAROGRAPHIC METHOD

REAGENTS AND APPARATUS

Dithizone soln.—Dissolve approximately 0.2 gram of dithizone in a liter of chloroform and keep the soln in a glass-stoppered Pyrex bottle in a cool place.

Ammonium citrate soln.—Prepare a stock soln of ammonium citrate by dissolving 113 grams of the salt in one liter of redistilled water. Adjust the *p*H of the soln to about 8.5 by adding 40–42 ml of conc. ammonium hydroxide. Then free the soln of zinc by extracting three times with the dithizone reagent, followed by washing three times with chloroform. Dilute to 1 liter 250 ml of the stock soln with redistilled water.

Supporting soln.—Make up a soln to be 0.1 *M* in ammonium acetate and 0.05 *M* in potassium thiocyanate.

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† Contribution from the Department of Agricultural Chemistry and Soils. Published by permission of the Director as Technical Paper No. 145.

Redistilled water.—Redistill all distilled water in a still made of Pyrex glass.

Chemical reagents.—Use C.P. reagents. Purify these reagents if they contain appreciable quantities of zinc.

Alkali fusion mixture.—One part Na_2CO_3 and two parts of K_2CO_3.

Glassware.—All glassware to be made of Pyrex glass, and washed with strong HCl and rinsed with redistilled water before use. Glass stoppered Pyrex bottles should be used, and if rubber stoppers are used these should be covered with waxed paper. The soln must not come in contact with rubber since rubber is a source of zinc contamination.

Polarograph.—The instrument used was of a photographic type having a drum capable of taking a full sheet of 8″×10″ enlarging paper, or a half-sheet, *i.e.*, 4″×10″. The electrolysis cell or polarizing cell had a capacity of 2.5 ml, and its anode was 0.5 ml of mercury at the bottom of the cell. The dropping electrode had a drop time of 5.83 seconds at the applied voltage of 1.2 volts, when the height of the mercury column was 18 inches. The cell was kept in a thermostat bath maintained at a temperature of 30°C., instead of the usual 25°C. The removal of oxygen dissolved in the supporting soln was accomplished by bubbling a slow stream of hydrogen thru the soln for three minutes. A Leeds and Northrup Type P galvanometer was employed, with sensitivity of 0.0008 microampere per mm, and shunted with a 4000-ohms resistance for damping. An electrolytic condenser of 2000-microfarads capacity connected to the circuit in the usual manner effectively cut down the galvanometer oscillations.

PROCEDURE

Weigh 0.5 gram of soil that has been ground to pass thru a 100-mesh sieve and transfer to a platinum crucible. Add 3 grams of $Na_2CO_3-K_2CO_3$ fusion mixture to soil and mix thoroly. Heat crucible until the fusion is completed (15–30 min.). Cool, and then place the crucible in a 400-ml beaker containing approximately 10 ml of distilled water. Add 10 ml of conc. HCl to the beaker to dissolve the cake. Crush the solid particles to facilitate soln of the cake and to free it from the crucible. After removal of the crucible, add 10 ml of conc. HCl, cover beaker with watch-glass, and digest on a hot plate until the sample is completely disintegrated. Remove watch-glass, rinse, and then evaporate the soln to dryness. Cool, moisten with 10 ml conc. HCl, let stand a few minutes, then add about 30 ml of 2 *N* HCl, digest at boiling point for 5 min. and decant hot liquid thru an ashless filter paper which had been washed previously with hot 2 *N* HCl. Redigest residue with 30 ml of 2 *N* HCl at boiling point for 5 min. and filter thru the same filter paper. Transfer all of the residue to the filter paper and wash with 2 *N* HCl and make up filtrate to 100 ml.

To a 125-ml separatory funnel containing about 40 ml ammonium citrate soln, add exactly 20 ml of the sample soln. Add two drops of phenolphthalein indicator and just enough conc. NH_4OH to impart a strong color to the soln. Add 10 ml of dithizone soln, stopper the funnel, and swirl the contents. After releasing the pressure, shake the funnel vigorously for 30 seconds and then allow layers to separate. Remove the glass stopper and draw off the chloroform layer into another 125-ml separatory funnel. Complete the extraction by shaking contents of the first separatory funnel with two 5-ml. portions of the dithizone soln.

To the dithizone soln in the second separatory funnel add exactly 30 ml of 0.1 *N* HCl and shake vigorously for 2 min. Allow the two layers to separate and then drain off the chloroform layer. Wash the aqueous layer with two 2-ml portions of chloroform, or until the chloroform layer is free of green coloration. Place exactly 25 ml of the aqueous soln containing the zinc in a 50-ml beaker and evaporate to dryness on a steam bath to free the residue of HCl. Cool the beaker and add exactly 10 ml of the supporting soln. This soln will dissolve the residue in 2 to 3 minutes.

Pour about 2 ml of the supporting soln containing the zinc into an electrolysis cell containing 0.5 ml of mercury to form the anode pool. Put the capillary (cathode) in position and pass hydrogen gas into the soln for three min., then obtain a polarogram by applying a voltage over the range of 0.8 to 1.2 volts. The step-height of the polarogram is proportional to the zinc concentration in the unknown soln. A standard curve is constructed by electrolyzing solns which have been prepared by diluting a standard zinc chloride soln with the ammonium acetate-potassium thiocyanate supporting soln. A set of polarograms are shown in Figure 1.

Exchangeable zinc can be determined by this method. A 100-gram sam-

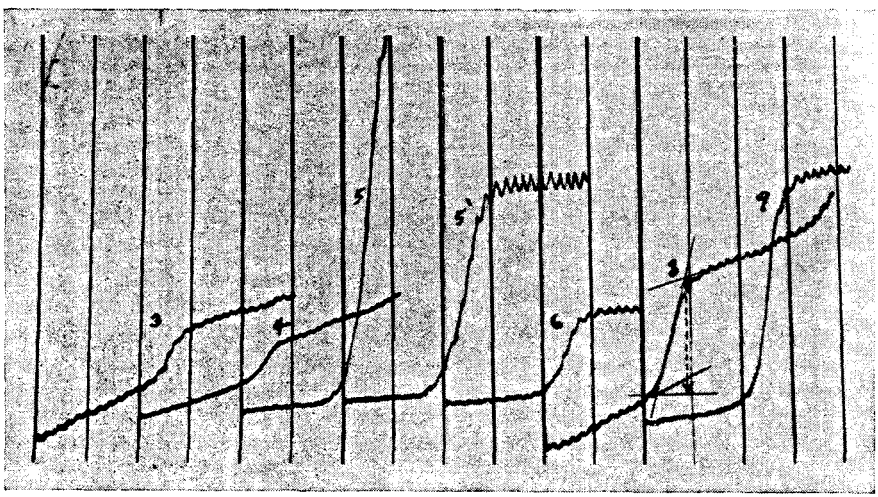

FIG. 1.—Polarographic waves of zinc extracted from soils. The method used for measuring the wave height is shown on curve 8. The curves 3, 4, and 8 were obtained at ½ maximum galvanometer sensitivity; the curve 9 at 1/5, the curves 5 and 6 at 1/10, and the curve 5′ at 1/20 the maximum galvanometer sensitivity.

ple of soil is extracted with neutral normal ammonium acetate, the extract is evaporated to dryness, the organic matter is oxidized, and the above procedure applied.

DISCUSSION

The alkali carbonate fusion mixture contained approximately 1.4 p.p.m. of zinc as an impurity. The concentration of zinc contributed by this reagent was less than 0.1 p.p.m. in the final electrolysis solution. The error introduced by this reagent can be compensated by running a blank determination for zinc in the chemical reagents.

The *p*H of the ammonium citrate solution before extraction with the dithizone solution should be 9.5–10.0 (3), but good results were obtained at any *p*H between 8.5 to 10.0. The proper *p*H value could be attained by

adding enough NH_4OH to impart a distinct pink color to the solution. According to Piper (2) zinc dithizonate is unstable in the presence of an excess of ammonia (*p*H 10.5).

It is advisable to have two similar cells. While the solution in one cell is being electrolyzed, the cell containing another solution may be purged of dissolved oxygen. The authors used a dozen cells, thereby eliminating the necessity for frequent rinsing.

At the sensitivity setting of "$\frac{1}{2}$," a solution containing 1.0 p.p.m. of

TABLE 1.—*The variation between replicated determination of zinc in solutions containing zinc, soils, and soil extracts obtained by leaching neutral normal ammonium acetate through soils*

TYPE OF MATERIAL TESTED	NUMBER OF DETERMINATION	RANGE OF ZINC CONCENTRATION FOUND*	AVERAGE OF DETERMINATIONS
		p.p.m.	*p.p.m.*
Solution containing 4.75 p.p.m. Zn	4	4.61– 4.78	4.72
Solution containing 1.65 p.p.m. Zn	4	1.57– 1.66	1.61
Soil 43 f	3	111.8 –116.0	114.27
Soil 43 g	3	118.9 –123.1	120.73
Soil 43 h	3	84.0 – 84.8	84.70
Exchangeable zinc (soil)	4	20.4 – 20.9	20.77
Exchangeable zinc (soil)	2	9.11– 9.30	9.20
Exchangeable zinc (soil)	3	0.50– 0.81	0.61

* Blank determination =0.02 p.p.m. of zinc.

zinc gave a wave height of about 2 cm. The concentration of zinc in soil samples analyzed thus far was 100–500 p.p.m. so that a 10–20 ml portion of the HCl solution, after the dithizone extraction, was sufficient to produce a wave height of 2–4 cm.

RECOVERY OF ZINC

The recovery of zinc added to soil samples was excellent by this method; in all cases more than 98 per cent of the added zinc could be recovered. The following result is typical: 100 micrograms of zinc were added to a soil containing 74.7 micrograms of zinc, making a total of 174.4 micrograms. The soil was analyzed in replicate by this method and found to contain 172.8 micrograms of zinc, which is equal to an average recovery of 98.4 per cent for the zinc added. The results of similar recovery experiments were consistently about 1.5 per cent too low, but this error was within the limits of accuracy expected for a polarographic method. When 1.0 ml. of a standard solution containing 20 micrograms of zinc was extracted and electrolyzed, the average of three analyses (19.8, 19.5, 19.8 micrograms) was 19.7 micrograms of zinc, or 98.5 per cent recovery.

The variation between replicated determinations for zinc by this method was found to be reasonably small. Table 1 gives data obtained from replicated determination of zinc in standard zinc solutions, soils, and in soil extracts containing the exchangeable zinc. The variation between the replicated determination was small except in the case of the soil extract containing only a trace of zinc.

The data in Table 2 was obtained from the analysis of a series of soil samples taken in a banana field in which certain areas are suspected of being zinc deficient. These data are evidence of the wide range of zinc

TABLE 2.—*A comparison of the total and exchangeable zinc content of Hawaiian soils from the Island of Oahu*

NATURE OF SOIL	pH	TOTAL* ZINC	EXCHANGEABLE* ZINC
		p.p.m.	*p.p.m.*
Established Zn-deficient soil	4.74	242.0	0.76
Suspected Zn-deficient soil†	7.20	291.0	0.61
Suspected mild Zn-deficient soil†	7.21	310.0	3.43
Soil producing normal plants	7.12	233.3	6.91
Soil producing normal plants	7.25	184.7	14.60

* Average of 3 replicated samples from area under investigation.
† From areas in a banana field in which plants have a physiological condition which is suspected of being due to zinc deficiency.

concentration to which this method has been found applicable. The range in this investigation was from 0.5 p.p.m. of exchangeable zinc to 310 p.p.m. total zinc in the soil.

The advantages of this method are that it is applicable to wide range of zinc concentration; it has a high degree of accuracy; and it is as rapid as the colorimetric method.

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DETERMINATION OF SOYBEAN FLOUR IN CEREAL PRODUCTS*

By V. E. MUNSEY (Food Division,† Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

Soybean flour is used in pancake mixes, wheat-soy macaroni products, bread, semi-sweet bakery products, and crackers. This study was undertaken to determine the application of a serological method for the estimation of soybean flour in cereal products. Ferguson, Racicat, and Rane,[1] and M. W. Hale,[2] have published similar serological methods for the determination of soybean flour in meat products. The preparation of an extract of a commercial full fat soybean flour, and the immunization of the rabbits, were carried out as described by Ferguson, Racicat, and Rane.[1] The rabbits were bled from the ear, the blood was clotted and centrifuged, and the serum was mixed with sufficient 7.5% solution of phenol to give a a strength of 0.25% phenol in the final serum. The serum was standardized against a control pancake mix of the usual composition containing wheat flour, sugar, defatted milk solids, salt, soda, mono-calcium phosphate and a known proportion of soybean flour. The method used on the control was identical with the method used on the unknown sample.

METHOD

Place 20 g of sample and 100 ml of 5% sodium chloride soln in a 250 ml flask with a few large beads and shake in a mechanical shaker for 1 hour. Filter thru folded filter paper. Transfer about 40 ml to a 50 ml tube, add 0.1 g takadiastase, mix, stopper, and allow to stand overnight at room temperature. Next morning filter thru folded filter, returning portions to filter to obtain a clear or slightly turbid filtrate. Set up a rack of 10 Wassermann tubes having the following dilution based on the control containing 2% of soybean flour:

Tube	1	2	3	4	5	6	7	8	9	10
Dilution	1:250	1:375	1:500	1:750	1:1000	1:1500	1:2000	1:3000	1:4000	1:6000

Tube 1 is the original undiluted extract. The actual dilutions are carried out by mixing 4 ml of the original undiluted extract with 2 ml of 0.5% sodium chloride for tube 2, and 2 ml of the original undiluted extract with 2 ml of 0.5% sodium chloride for tube 3. The remaining dilutions in even and odd numbered tubes are made by mixing equal parts from tube 2 and 3, respectively, with 0.5% sodium chloride so that each succeeding even and odd numbered tube has ½ the concentration of the preceding tube. Place 1 ml of the appropriate dilution in the Wassermann tube, add 0.2 ml of rabbit serum, shake to mix, and incubate at 56°C. for 2½ hours. Then place the tubes in the refrigerator to remain overnight. The farthest tube to the left showing a distinct precipitate is the indicator tube, that is, the

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[1] *This Journal*, 25, 533–537, 1942.
[2] *Food Research*, 10, 1–6, 1945.

one which indicates the titer of the serum. By adjusting the proportion of serum, by dilution of the serum, or of the extract, the ratio of antibody to antigen can be adapted to a rather wide percentage range of soybean flour. Since the amount of soybean flour in the series of control tubes are multiples of the 2% in the control, the estimation of soy flour in the unknown is quite simple. It is only necessary to divide the dilution of the indicator tube for the unknown sample by that of the indicator tube of the known soybean sample, treating the denominators as whole numbers. This result multiplied by 2 will give the percentage of added soybean flour. Some of the rabbit serum gave better results diluted with an equal volume of physiological salt solution, and most of the experiments were therefore carried out with such a (1+1) serum.

EXPERIMENTAL

An illustration of typical results on a series of pancake mixes containing known amounts of the same soybean flour follows:

	DILUTIONS									
% SOY FLOUR ADDED	1/250	1/375	1/500	1/750	1/1000	1/1500	1/2000	1/3000	1/4000	1/6000
1	—	—	++	++	++	++	++	+	+	—
2	—	—	—	—	++	++	++	++	++	+
3	—	—	—	—	—	++	++	++	++	++
4	—	—	—	—	—	—	++	++	++	++

Note that the estimated amounts are 1.0, 2.0, 3.0, and 4.0% respectively, corresponding exactly to the amounts of soybean flour actually present.

In order to test the variation in commercial soybean flours of different types and processing treatments, a series of 12 pancake mixes, all containing 2.5% soybean flour, were examined with the results shown in Table 1.

These results indicate that there is sufficient variation in the titer of commercial soybean flours to give considerable differences in the estimated amount of soybean flour in unknown products. While the above flours were not run in duplicate with this serum, a sufficient number of them have been run against serum of a different titer to confirm this variation in commercial soy flours.

Four pancake mixes, containing amounts and types of soybean flour unknown to the analyst, were analyzed in duplicate. The soy flour used as a control was different from the soybean flours in each of these unknown mixtures.

In general, the estimated amount is not in close agreement with the actual amount of soy flour present.

% SOYBEAN FLOUR ADDED	% SOYBEAN FLOUR ESTIMATED
7.0	10.0
	10.0
2.5	2.0
	2.0
7.0	2.0
	2.0
3.0	1.0
	1.0

SOYBEAN FLOUR IN MACARONI

A wheat-soy macaroni containing 10 per cent soy flour was analyzed by the pancake mix procedure and a result of 6.7 per cent soy flour was obtained. The control soy flour was not the same as the soy flour in the macaroni.

TABLE 1.—*Pancake mixes containing 2.5% soybean flour of different types from different manufacturers*

			% ESTIMATED AMOUNT OF SOYBEAN FLOUR
Sample	1	Extracted soybean flour	5.0
	2	Extracted soybean flour	5.0
	3	Extracted soybean flour	3.3
	4	Expeller soybean flour	1.3
	5	Expeller soybean flour	2.5
	6	Expeller soybean flour	1.7
	7	Expeller soybean flour	1.3
	8	Full fat soybean flour	2.5
	9	Full fat soybean flour	2.5
	10	Full fat soybean flour	2.5
	11	Full fat soybean flour	3.3
	12	Full fat soybean flour	1.3

SOYBEAN FLOUR IN BREAD

Breads were made containing 3, 5, 10 and 15 per cent of 6 different commercial soy flours, but not all 6 samples of soy flour were incorporated at each percentage level. The breads were analyzed by the pancake mix procedure, using both the fresh crumb and the ground air-dried bread. The air-dried crumb was found preferable, and also a 10 g sample instead of 20 g. The results obtained were considerably below the actual amount of soy added, indicating that the soy protein may be denatured during

bread production. A variation in commercial soy flours was also observed in the bread similar to that found in the pancake mixes. A few typical results are given below:

% SOY FLOUR ADDED TO BREAD	% SOY FLOUR ESTIMATED
3	2.2
3	1.7
3	0
5	2.2
10	4.0
15	4.0

CONCLUSION

This serological method is satisfactory for the determination of soybean flour in pancake mixes and wheat-soy macaroni if the soybean flour used in their production is available for a control. When there is no knowledge of the soy flour used in these products, the method affords only an approximate estimation. The applicability of the method to bread containing added soy flour is extremely limited.

Grateful acknowledgement is made to Geoffrey Woodard and Ernest Hagan of the Division of Pharmacology, Food and Drug Administration, for the immunization of the rabbits, and to Dr. E. J. Coulson, Allergen Research Division, Bureau of Agricultural and Industrial Chemistry, U.S.D.A., for advice on this work.

THE ANTIRACHITIC POTENCY OF PURE CRYSTALLINE VITAMIN D_3 IN COMPARISON WITH THE U.S.P. REFERENCE COD LIVER OIL WHEN ASSAYED BY THE CHICK METHOD

By J. WADDELL AND G. H. KENNEDY (Biological Laboratory, E. I. duPont de Nemours & Co., New Brunswick, N. J.)

INTRODUCTION

The Second International Standardisation Conference held in 1934 (1) recommended that the special solution of irradiated ergosterol in the possession of the National Institute for Medical Research, London, be continued as the international standard for vitamin D. Although it was also recommended that, if the supply of this solution became exhausted or if it became unsatisfactory for any reason, crystalline vitamin D_2 (calciferol) was to be used as the standard at a value of 40×10^6 I.U. per gram of the pure material, this change apparently has never been made.

It has long been recognized that any vitamin D standard based on D_2 is unsatisfactory in at least one important respect: it is comparatively

ineffective on certain species, such as poultry, for which vitamin D is an especially essential dietary constituent. This latter consideration formed one of the main reasons for establishing subsidiary standards, such as the U.S.P. reference cod liver oil, which could serve not only for the testing of pharmaceutical and food preparations by the rat assay method, but also for the standardization of vitamin D supplements for poultry by the chick method.

The procedure followed in determining the vitamin D potency of the present issue of the U.S.P. reference cod liver oil (No. 2) has been described (2). The vitamin D value of 115 U.S.P. units per gram was assigned on the basis of reports from 18 laboratories which assayed the new oil by the U.S.P. (rat) method against samples of the international standard; and of reports from 17 laboratories which assayed the new oil by the A.O.A.C. (chick) method in comparison with the old reference cod liver oil (No. 1). The average values of these two series of assays were in good agreement (114 and 119 units, respectively) and seemed to justify the assigned value. Therefore, the label of the reference cod liver oil states that the U.S.P. unit of vitamin D is identical with the international unit; and the A.O.A.C. chick unit of vitamin D is defined (3) as the biological activity for the chick of one U.S.P. unit of vitamin D from the reference cod liver oil. The U.S.P. reference oil No. 2 is the same as that distributed in Canada as Canadian reference cod liver oil.

Since crystalline vitamin D_3 was first isolated from irradiated 7-dehydrocholesterol by Schenck (4) its possible advantages as a standard for vitamin D have been considered. The use of such a substance, with maximum antirachitic potency for all species and available in pure crystalline form, would remove the drawbacks of a standard based on D_2 and the difficulties of procuring and standardizing subsidiary standards such as reference cod liver oil. Thus in 1939 (5) nine British laboratories collaborated in comparing the antirachitic potency of a German sample of crystalline D_3 with that of vitamin D_2 (calciferol) using the rat as the test animal. This study was undertaken in preparation for the intended third meeting of the International Standardisation Conference, which could not be held. It was found in this test that, for the rat, both substances had equal antirachitic potency and it was concluded that the potency of crystalline vitamin D_3 was 40×10^6 I.U. per gram. The sample of vitamin D_3 used had the following constants, which would indicate a purity somewhat less than that of samples described later by American workers (6) or of those available to us in the tests to be described.

M.P. 78°–82°C.

$[\alpha]^{25}_{546.1} = +125°$

$[\alpha]^{25}_{D} = +105°$ (ethanol)

$E^{1\%}_{1cm\lambda 266m\mu} = 424$ (ethanol) [ϵ (calculated) = 16,280]

In 1940 the British Standards Institution published Specification 911 (7) outlining their method for the biological assay of vitamin D_3 by the use of the chick. The Provisional Standard Preparation of vitamin D_3, the vitamin D standard to be used in such assays, was described as a solution of pure crystalline vitamin D_3 in olive oil of a concentration such that 1 mg. of the resulting solution contained 0.000025 mg. of the crystalline vitamin. This amount was designated as 1 B.S.I. unit, which was equivalent to placing a value of 40×10^6 B.S.I. units per gram on the vitamin D_3. The vitamin D activity of fish oils or concentrates intended for use in poultry or livestock feeds, when compared with the Provisional Standard Preparation, could then be expressed in B.S.I. units. The quality of the crystalline D_3 used in the preparation of this standard is not described, but it appears to have been either the German specimen mentioned above or material of similar purity (8).

We have been informed that since the B.S.I. provisional standard preparation was issued, certain laboratories have noted distinct differences in the calculated potency of samples when they were assayed in comparison with both the reference cod liver oil and the B.S.I. standard. Personal communications from investigators in Canada,[1] where both standards had some currency, indicated that the B.S.I. unit represented distinctly greater antirachitic activity for the chick than the A.O.A.C. unit. This meant, therefore, that the potency of crystalline D_3 could be expected to be greater than 40×10^6 A.O.A.C. units per gram; a conclusion which earlier work in this laboratory with purified D_3 preparations would support.

Because of the need for more extensive and more precise information on the antirachitic potency of pure vitamin D_3 in comparison with the U.S.P. reference cod liver oil, and also as part of a general study aimed at the improvement of the A.O.A.C. chick assay procedure, the Executive Committee of the Animal Vitamin Research Council approved the carrying out of a collaborative assay of these two substances among a group of its members. This experiment was carried out during 1943 under the general supervision of one of us (J. Waddell). A total of seven laboratories, including our own, participated, the number being limited, since we wished to supply each laboratory with sufficient of the crystalline D_3 (rather than a solution of it) for the purposes of the test. Each collaborator was asked to submit to us a copy of his test data together with his own estimate of potency.

The availability of the crystalline D_3 presented us the opportunity of including it in a subsequent series of chick assays which are regularly carried

[1] In 1942 Dr. A. R. G. Emslie of Ottawa, Canada, was kind enough to send us a summary of some unpublished data from comparative assays of the two standards. Also, Prof. J. Biely of the University of British Columbia in 1944 sent us the protocols of a similar extensive assay. The data from both investigators indicated a value of between 1300 and 1400 A.O.A.C. units per gram of the B.S.I. standard solution (*i.e.* the B.S.I. unit was 30 to 40% more active than the A.O.A.C. unit.

out in this laboratory and which, of course, regularly include groups receiving the reference cod liver oil. In this way we obtained an additional total of 16 comparisons of this sample of D_3 with reference cod liver oil.

Somewhat later we obtained another sample of crystalline vitamin D_3 prepared in the Du Pont laboratories and this also we were able to compare with reference cod liver oil in a further series of 11 chick tests.

It is the purpose of this report to present the data from these various comparisons and the quantitative interpretation which was made of them.

EXPERIMENTAL

Samples Used.

The first sample of crystalline vitamin D_3 was a generous gift of the Winthrop Chemical Co., Rensselaer, N. Y., through the courtesy of Drs. Barlow and Huber. It was given by us the identification number of 4–188. It was received in the form of a number of sealed evacuated amber-glass ampoules each containing approximately 10 mg of material.

This sample was reported by Dr. Huber as possessing the following constants:

$$\text{M.P.} \qquad 82.5\text{–}83.5°\text{C. (uncorr.)}$$
$$[\alpha]_D^{20} = +82.9° \text{ (acetone)}$$
$$\epsilon_{\lambda 265 m\mu} = 18{,}600 \pm 200$$

These constants were checked in the Du Pont laboratory and found to be essentially correct. Similar values for the specific rotation and for molecular extinction were found in the E. R. Squibb & Sons laboratory.

The second sample of crystalline vitamin D_3 which we used was prepared by Dr. W. C. Meuly in the Chemical Laboratory (New Brunswick Works) of this company. It was identified as 64–122 and exhibited the following constants which indicate a product of excellent purity.

$$\text{M.P.} \qquad 83.5\text{–}86.5°\text{C. (uncorr.)}$$
$$[\alpha]_D^{25} = +111° \text{ (ethanol)}$$
$$+84.8° \text{ (acetone)}$$
$$+52.7° \text{ (chloroform)}$$
$$\epsilon_{\lambda 265 m\mu} = 18{,}680$$

The samples of U.S.P. reference cod liver oil No. 2 used in all of the tests reported were obtained, as needed, by each laboratory directly from Dr. E. Fullerton Cook, Chairman, Committee of Revision, U.S. Pharmacopoeia, Philadelphia.

Technique of Chick Assays.

All of the assays reported in this paper, with the exceptions noted below,

were carried out essentially by the A.O.A.C. Method (3). Single comb white Leghorns, generally received in the laboratory on the second day of life, were used throughout. In all of the tests in this laboratory, and in some of the collaborating laboratories, the chicks were obtained from one commercial hatchery which in turn used eggs from a single flock to hatch chicks for this purpose. At certain seasons of the year the chicks were all cockerels, at other seasons they included both sexes. The chicks when received were divided into as uniform groups as possible, containing generally 18 to 20 birds, and then were fed the supplemented diets for the next 21 days. The brooders had wire screen bottoms and were supplied with auxiliary heaters. In some laboratories, including our own, the brooders were housed in rooms with controlled temperature and humidity.

The A.O.A.C. rachitogenic diet for chicks has the following composition: ground yellow corn, 58; wheat flour middlings, 25; crude domestic acid precipitated casein, 12; calcium phosphate (precipitated), 2; dried yeast, 2; iodized salt, 1. To each kg. of this diet is added 0.2 gm of $MnSO_4 \cdot 4H_2O$. It is the usual custom in assaying oil-soluble materials to use 99 per cent of the above diet and 1 per cent of a vegetable oil carrying the calculated amount of supplement.

In one of the assays carried out in the collaborative experiment (laboratory 1) a modified A.O.A.C. basal diet was used and the chicks were "depleted" for 10 days before the assay was started.

In the collaborative experiments and in the first series of assays in this laboratory the criterion of response to the different doses of supplements was the ash content of the cleaned, extracted tibiae, expressed as a percentage of the dried fat-free bones. A composite sample, consisting of a single bone from each chick, was analyzed to obtain the group response.

In the second series of assays in this laboratory the technique was changed only to the extent of using for the bone ash determination the distal portion of the middle toe from one foot, carefully severed at the first joint, in place of the cleaned tibia. The use of the toe in vitamin D assays was first reported by Baird and MacMillan in 1942 (9). Several investigators (10, 11, 12) have since reported that the ash content of the toe was equally as satisfactory as that of the tibia for estimating response to vitamin D, and unpublished observations in this laboratory have confirmed these findings.

Collaborative Assays.[2]

Six laboratories, in addition to our own, participated in this test. We

[2] In addition to distributing sample 4–188 among American laboratories several ampoules were sent to Sir Jack Drummond in 1943 on one of his visits to Washington. He delivered the material to Dr. S. K. Kon, Cod Liver Oil (Poultry) Standardization Laboratory, Shinfield, England, for comparative assay against the B.S.I. Provisional Standard Preparation. Dr. Kon carried out two assays in the autumn of 1943 and three more 11 months to a year later, using the original oil dilutions. In these tests he found sample 4–188 to exhibit a potency appreciably in excess of 40×10^6 B.S.I. units per gram. We were privileged to receive complete protocols from Dr. Kon and we presume his results will be published as part of a more extensive study which he and his associates carried out.

are indebted to the following investigators for their cooperation:

F. D. Baird, National Oil Products, Harrison, N. J.
A. Black, E. R. Squibb and Sons, New Brunswick, N. J.
H. J. Cannon, Laboratory of Vitamin Technology, Chicago, Ill.
E. W. McChesney, Winthrop Chemical Co., Rensselaer, N. Y.
B. L. Oser, Food Research Laboratories, Long Island City, N. Y.
T. D. Sanford, F. E. Booth Co., San Francisco, Calif.

Each investigator was supplied with a number of ampoules of the crystalline vitamin D_3, sample 4–188, and, hence, each made his own weighing of the sample and all subsequent dilutions in oil. An assumed potency of 45,000 A.O.A.C. units per mg., recommended by us, was used in calculating all doses of the vitamin D_3.

The doses of reference cod liver oil and of the crystalline D_3 which were fed, and the bone ash responses obtained by each laboratory, are shown in Table 1. The estimate of potency of the D_3 by each collaborator is shown in the second column of Table 4. One investigator was unwilling to make a quantitative estimation from his results.[3]

Additional Assays on Sample 4–188.

In order to obtain additional data on the antirachitic activity of this sample in comparison with the reference cod liver oil we included it as an additional "unknown" in a series of chick assays in this laboratory. A total of 16 such comparative tests was carried out over a period of 11 months and in all of these the same oil solution of the vitamin D_3 was used.

The bone ash responses of the different groups which received graded doses of both the reference oil and the vitamin D_3 in this series are shown in Table 2. The calculated potency of the D_3 from this series is presented in Table 5. The numerical procedure followed in these calculations is discussed below.

Assays on Sample 64–122.

The availability in our laboratory of another sample of crystalline vitamin D_3 of high purity induced us to carry out a further series of comparisons with reference cod liver oil. A total of 11 assays of this material was completed over a period of 6 months. In Table 3 the percentage of ash of the extracted toes of the various groups in these tests is recorded. The estimated potency of this sample of vitamin D is shown in Table 6.

Interpretation of Assays.

The interpretation of the data of all the assays, other than the results as given by the collaborators in column 2 of Table 4, is based upon methods

[3] At the annual meeting of the Animal Vitamin Research Council held in Washington, October 26, 1944, a preliminary report (23) of the results of the collaborative assays was made by one of us (G. H. Kennedy). We believe that the mean value for the potency of vitamin D_3 now shown in Table 4 is a more acceptable one than the 49,222 units per mg suggested at that time.

TABLE 1.—*Responses of all groups in the collaborative assays of sample 4–188 as percentage of ash of extracted tibiae*

UNITS* VITAMIN D PER 100 GM. DIET	SOURCE OF VITAMIN	
	REF. C.L.O.	SAMPLE 4–188 IN VEGETABLE OIL
	Per cent ash	*Per cent ash*
	Lab. No. 1	
0	30.97	
5.0	33.40	34.40
7.5	37.28	35.95
10.0	37.30	38.26
12.5	38.95	41.28
15.0	41.05	42.70
17.5	42.80	44.66
20.0	43.80	45.70
30.0	45.10	46.38
	Lab. No. 2	
0	29.31	
3	28.67	28.86
6	30.79	31.80
9	34.37	36.76
12	38.28	40.02
	Lab. No. 3	
0	32.9	
4	33.3	33.8
8	35.3	36.7
8	35.0	36.1
12	37.4	39.3
16	40.2	41.6
16	40.4	42.1
20	39.7	40.8
	Lab. No. 4	
0	32.3	
2	34.2	32.7
4	34.6	34.9
8	35.3	35.9
12	39.1	39.5
16	41.2	41.6
20	39.8	42.2
24	42.0	43.2
32	42.7	42.6
	Lab. No. 5	
0	30.13	
6	30.62	
9	32.64	
12	33.26	
18	35.63	
24	39.96	
11.7		33.24
13.5		34.74
15.9		34.83
19.3		36.06
	Lab. No. 6†	
0	31.4	
2	33.2	33.3
4	33.3	39.9
8	33.6	38.8
12	39.8	40.2
16	38.9	42.6
24	43.6	44.4
32	44.4	44.7
	Lab. No. 7	
0	31.42	
2	33.08	32.99
4	33.60	33.03
8	34.49	33.92
12	37.35	38.62
16	39.30	40.09
24	40.24	43.00

* Calculated on basis of 115 units per gram for U.S.P. Reference Cod Liver Oil No. 2 and 45,000 units per milligram for Sample 4–188.
† Means of both left and right tibiae.

TABLE 2.—*Responses of all groups in Du Pont assays of sample 4–188 as percentage of ash of extracted tibiae*

UNITS* VITAMIN D PER 100 GM. DIET	SOURCE OF VITAMIN	
	REF. C.L.O.	SAMPLE 4–188 IN VEGETABLE OIL
	Per cent ash	*Per cent ash*
	Expt. No. 98	
0†	32.9	
4	33.3	33.8
8	35.3	36.7
8	35.0	36.1
12	37.4	39.3
16	40.2	41.6
16	40.4	42.1
20	39.7††	40.8††
	Expt. No. 105	
0‡	33.0	
5	34.5	34.6
7.5	35.7	—
10	37.7	38.2
12.5	39.9	—
15	42.3	41.5
20	41.5††	43.5
	Expt. No. 106	
0‡	31.9	
5	33.0	34.0
10	36.1	37.2
12.5	36.2	—
15	37.4	40.7
20	41.1	44.1
	Expt. No. 107	
0‡	32.3	
5	34.2	32.9
7.5	34.8	—
10	35.9	34.9
12.5	34.6	—
15	36.3	39.1
20	38.1	41.9
	Expt. No. 108	
0‡	31.9	
5	32.5	33.8
10	33.1	35.3
12.5	33.5	—
15	36.6	37.6
20	37.9	40.7

UNITS* VITAMIN D PER 100 GM. DIET	SOURCE OF VITAMIN	
	REF. C.L.O.	SAMPLE 4–188 IN VEGETABLE OIL
	Per cent ash	*Per cent ash*
	Expt. No. 109	
0	33.5	
4	35.8	34.2
6	37.2	37.6
9	40.1	39.0
13.5	42.9	44.0
20.25	44.8	44.2
	Expt. No. 110	
0	31.5	
5	34.5	35.0
10	37.3	39.7
12.5	39.1	—
15	40.6	41.7
17.5	41.1	—
20	41.9	43.5
	Expt. No. 111	
0	33.3	
4	35.5	35.4
6	35.5	35.4
9	38.4	37.6
13.5	41.4	39.6
20.25	42.7	43.6
	Expt. No. 112	
0	32.4	
4	33.0	35.5
6	33.6	34.1
9	34.8	33.1
13.5	38.1	42.3
20.25	41.9	42.9
	Expt. No. 113	
0	33.6	
4	37.5	35.1
6	37.2	37.3
9	39.8	38.9
13.5	41.7	42.4
20.25	43.4	41.9

For footnotes, see page 198.

TABLE 2.—(*continued*)

UNITS* VITAMIN D PER 100 GM. DIET	SOURCE OF VITAMIN		UNITS* VITAMIN D PER 100 GM. DIET	SOURCE OF VITAMIN	
	REF. C.L.O.	SAMPLE 4–188 IN VEGETABLE OIL		REF. C.L.O.	SAMPLE 4–188 IN VEGETABLE OIL
	Per cent ash	*Per cent ash*		*Per cent ash*	*Per cent ash*
	Expt. No. 114			*Expt. No. 118*	
0	33.6		0	35.2	
6	36.7	36.2	4	34.5	35.3
9	38.6	38.4	8	38.5	37.6
13.5	42.2	41.0	12	40.3	—
17	43.1	—	16	44.4	40.7
20.25	44.8	43.9	24	43.9††	43.7††
			32	43.1††	44.8††
	Expt. No. 115			*Expt. No. 119*	
0	34.8		0	36.2	
6	37.5	37.1	6	38.6	40.1
9	41.1	39.2	9	40.8	39.0
13.5	42.4	42.7	13.5	41.3	43.8
17	43.1	—	17	44.3	—
20.25	44.8	42.3	20.25	43.3††	43.2††
	Expt. No. 117			*Expt. No. 120*	
0	32.9		0	33.6	
6	37.6	36.4	6	34.3	33.4
9	38.9	37.4	9	37.6	37.6
13.5	42.6	42.7	13.5	39.5	41.8
17	43.6	—	17	41.3	—
20.25	44.3	42.7	20.25	43.0	42.0

* Calculated on basis of 115 units per gram for Reference Cod Liver Oil No. 2 and 50,000 units per milligram, except as noted, for Sample 4–188.
† Calculated on basis of 45,000 units per milligram for Sample 4-188.
‡ Calculated on basis of 47,500 units per milligram for Sample 4-188.
†† These values were omitted from the computations.

for the graded dose response type of assay, to which the chick assay for vitamin D belongs. Gaddum (13), Irwin (14), Bliss (15, 16), and others, have been responsible for developing the mathematical background of these methods and demonstrating their applicability to a wide range of assays carried out with a variety of drugs and using various laboratory animals. Application in whole or in part of the principles described by the above investigators appears in several places in the literature with respect to the assay of vitamin D with chicks (7, 11, 17).

When the responses in a biological assay are plotted against their respective doses, the relation between the responses and the logarithms of the doses will approximate a straight line in a majority of cases unless responses are obtained which are too close to the minimum or maximum

TABLE 3.—*Responses of all groups in Du Pont assays of sample 64–122 as percentage of ash of extracted toes*

UNITS* VITAMIN D PER 100 GM. DIET	SOURCE OF VITAMIN	
	REF. C.L.O.	SAMPLE 64–122 IN VEGETABLE OIL
	Per cent ash	*Per cent ash*
	Expt. No. 139	
0	8.14	
5	9.00	8.87
10	10.08	10.08
15	10.76	—
20	12.21	13.44
	Expt. No. 140	
0	8.40	
5	9.12	8.70
10	9.97	9.55
20	11.63	13.00
	Expt. No. 141	
0	7.55	
5	8.79	—
5	8.58	9.03
10	9.89	—
10	10.08	10.37
20	11.92	—
20	11.48	12.62
	Expt. No. 142	
0	7.22	
5	8.69	—
5	8.00	8.70
10	10.03	—
10	9.89	9.49
20	12.17	—
20	12.48	13.46
	Expt. No. 143	
0	4.33	
5	7.67	—
5	7.94	8.04
10	8.50	—
10	8.85	9.50
20	11.15	—
20	12.06	12.16

UNITS* VITAMIN D PER 100 GM. DIET	SOURCE OF VITAMIN	
	REF. C.L.O.	SAMPLE 64–122 IN VEGETABLE OIL
	Per cent ash	*Per cent ash*
	Expt. No. 144	
0	7.72	
5	8.58	—
5	8.25	8.54
10	9.31	—
10	9.99	10.22
20	13.02	—
20	11.82	13.20
	Expt. No. 145	
0	8.27	
5	8.44	—
5	8.40	8.53
10	10.25	—
10	10.09	10.24
20	13.22	—
20	13.59	13.89
	Expt. No. 146	
0	7.50	
5	8.41	—
5	8.34	8.65
10	10.34	—
10	9.01	9.84
20	12.38	—
20	12.69	13.21
	Expt. No. 148	
0†	8.45	
5	9.11	9.76
10	10.32	10.53
20	12.59	13.10
	Expt. No. 149	
0†	8.70	
5	8.66	8.52
10	9.46	10.15
20	11.38	12.39
	Expt. No. 151	
0†	8.04	
5	9.80	9.04
10	10.36	10.44
20	12.46	12.96

* Calculated on basis of 115 units per gram for Reference Cod Liver Oil No. 2 and 50,000 units per milligram, except as noted, for Sample 64–122.
† Calculated on basis of 55,000 units per milligram for Sample 64–122.

TABLE 4.—*Estimated potency of sample 4–188 in millions of A.O.A.C. units per gram from collaborative assays*

COLLABORATOR NO.	AS REPORTED BY COLLABORATOR	ASSAYS COMPUTED SEPARATELY	ASSAYS COMPUTED WITH COMBINED STATISTICS*	
	Potency	*Potency S.E.*	*Potency S.E.*	*Confidence Limits (P=0.05)*
1	50.800	53.2 ± 3.6	53.1 ± 4.1	45.4– 62.6
2	51.500	54.0 ± 7.9	54.0 ± 5.8	43.2– 68.0
		Weighted Mean and S.E. 53.4 ± 3.34		
3	58.000	58.0 ± 5.9	62.0 ±11.4	43.0– 90.3
4	45.450	50.9 ± 8.2	50.4 ± 8.6	35.8– 71.4
5	40.470 ±2.055	39.1 ± 4.1	40.1 ± 9.2	25.2– 63.6
6	—	81.8 ±20.7	82.2 ±15.3	56.9–120.5
7	43.500	53.1 ±13.8	52.2 ±10.3	34.9– 78.2
		Weighted Mean and S.E. 57.0 ± 7.53		
		Grand Weighted Mean and S.E. 54.7 ± 2.82		

* Computed using as statistics for collaborators 1 and 2, b = 16.776805, s = 1.103; and for collaborators 3, 4, 5, 6, and 7, b = 9.333660, s = 1.386.

TABLE 5.—*Estimated potency of sample 4–188 in millions of A.O.A.C. units per gram from Du Pont assays*

ASSAY NO.	ASSAYS COMPUTED SEPARATELY	ASSAYS COMPUTED WITH COMBINED STATISTICS*	
	Potency S.E.	*Potency S.E.*	*Confidence Limits (P=0.05)*
98	54.0 ± 5.9	57.2 ± 6.6	45.5–72.1
105	49.6 ± 3.9	51.8 ± 7.0	39.7–67.7
106	47.5 ± 9.6	72.9 ± 9.9	55.9–95.5
107	31.8 ± 1.0	60.1 ± 7.8	46.5–77.8
108	80.1 ±18.1	71.5 ± 9.6	54.8–93.6
109	47.2 ± 5.0	46.9 ± 5.9	36.4–60.2
110	64.3 ± 2.9	64.7 ± 8.4	50.1–83.7
111	46.4 ± 5.9	46.7 ± 5.9	36.3–60.0
112	61.7 ± 9.9	63.2 ± 8.0	49.2–81.4
113	41.5 ± 5.6	43.3 ± 5.5	33.6–55.6
114	45.4 ± 1.7	43.9 ± 5.9	33.6–57.3
115	41.0 ± 5.0	41.9 ± 5.7	32.1–54.7
117	41.7 ± 4.9	40.9 ± 5.5	31.2–53.3
118	41.3 ± 8.2	41.8 ± 6.4	30.8–56.6
119	59.3 ±16.2	59.1 ± 9.0	43.7–80.1
120	51.2 ± 4.6	50.4 ± 6.8	38.6–65.7
	Weighted Mean and S.E. 52.7 ±2.66		

* b = 12.763105, s = 1.1097.

possible response. Considered graphically, the distance (M) on the dosage axis between two linear and parallel dosage response curves, determined from two different preparations, measures the difference in units of log dose between the two curves for the same response. This relationship between responses may be used to express the relative activity or potency of one preparation in terms of the other; for example, an unknown in terms of a standard preparation whose potency is arbitrarily fixed.

The relative potency of an unknown preparation in terms of a standard

TABLE 6.—*Estimated potency of sample 64–122 in millions of A.O.A.C. units per gram from Du Pont assays*

ASSAY NO.	ASSAYS COMPUTED SEPARATELY	ASSAYS COMPUTED WITH COMBINED STATISTICS*	
	Potency *S.E.*	*Potency* *S.E.*	*Confidence Limits (P=0.05)*
139	61.5 ± 12.0	61.2 ± 9.7	44.5–84.2
140	54.2 ± 8.3	53.9 ± 9.2	38.3–76.0
141	63.4 ± 5.4	60.7 ± 8.9	45.2–81.7
142	56.3 ± 8.2	56.4 ± 8.3	42.0–75.8
143	60.6 ± 9.5	60.5 ± 8.9	45.1–81.3
144	58.8 ± 8.4	59.5 ± 8.8	44.3–80.0
145	53.1 ± 5.1	54.1 ± 8.0	40.3–72.7
146	56.4 ± 8.5	57.0 ± 8.4	42.5–76.7
148	66.3 ± 11.0	64.7 ± 11.0	46.1–91.1
149	64.6 ± 10.0	63.1 ± 10.7	44.9–88.8
151	53.6 ± 10.7	53.9 ± 9.2	38.3–75.7
	Weighted Mean and S. E.	58.4 ± 2.75	

* b = 6.528346, s = 0.5896.

in the graded dose response type of assay may be computed from the equation

$$M = \bar{x}_s - \bar{x}_u - \frac{\bar{y}_s - \bar{y}_u}{b_c}, \tag{1}$$

where $\bar{x}_s$, $\bar{x}_u$ and $\bar{y}_s$, $\bar{y}_u$ represent the mean dosages and responses of the standard and unknown respectively, and b_c is the (combined) slope or measure of response per unit of dose.

The reliability of the method of assay depends upon (a) whether the computed dosage-response lines represent the data adequately (measured by s in equation 2 below) and (b) a minimal ratio, between the standard deviation and the slope of the lines. The following equation for obtaining the standard error of the estimate of potency from the data of a single assay is based upon the above considerations,

$$s_M = s/b_c \sqrt{\frac{1}{N_s} + \frac{1}{N_u} + \frac{(\bar{y}_s - \bar{y}_u)^2}{B_c^2}}. \tag{2}$$

In equation (2) s is the standard deviation of the responses (obtained from the mean square for error) which expresses the departure from the calculated response curve, N_s and N_u are the numbers of observations (groups of chicks) fed the standard and unknown, B_c^2 is the variance accounted for by the slope

$$B_c^2 = \frac{S^2[xy]}{S[x^2]}$$

and the other symbols are the same as in equation (1). The ratio s/b_c is denoted by the symbol λ.

An example of the data from a chick assay, and of the computations leading to the values represented by the symbols appearing in equations (1) and (2) for M and s_M, is detailed in a recent publication (18).

In computing the standard deviation from each assay separately, the number of independent observations (degrees of freedom) was considered to be 3 less than the total number of responses of the groups fed the standard and unknown. This procedure was based on a study of the collaborative data. In an analysis of variance of the slopes of the individual response curves, the mean square for the differences between the groups fed the standard and those fed the unknown, was well within the limits expected from the sampling error. Hence, the two sources of vitamin D might be considered as qualitatively the same. This assumption had the effect of increasing the quantity of data on which to base the estimate of error by one observation in each assay. The computation of the standard deviation is given in the reference (18). The same procedure was followed in all computations of the error when the assays were computed individually (columns 2 of Tables 5 and 6). It was justified in each group of assays by the non-significance of the "F" values which tested the departure from parallelism of the dosage-response curves for the standard and for the unknown in their respective analyses of variance.

The results obtained by substituting the data of each single assay in equations (1) and (2) are given in column 3 of Table 4 and columns 2 of Tables 5 and 6.

No weighting of the responses was attempted for differences in numbers of chicks composing the various groups.[4]

As stated above, the reliability of an assay is directly proportional to the ratio of the standard deviation (s) and the slope (b_c). The averages of the bone ash values resulting from specific doses of vitamin were not stable from one assay to another but the standard deviation and slope of the individual assays generally did not vary more than would be expected

[4] In the collaborative assay, collaborators 2, 4, and 7 failed to report the numbers of chicks. Collaborators 1, 3, 5, and 6 reported the numbers of chicks in the groups as 14–16, 19–21, 14–17, and 18–20 respectively. In all the other assays reported here, the numbers of chicks per group were 17–20 except for assay 113 of vitamin D, 4–188, where the chicks numbered 11 or 12.

from the errors of random sampling. If this "state of statistical control" were to hold over a series of assays on the same sample, values could be obtained which would have increased reliability. In the present report the statistics for slope and standard deviation were combined for each of the three groups of assays after examining their "homogeneity." The information on slopes was pooled to minimize the confidence limits in Tables 4, 5, and 6. It was recognized, of course, that the use of a common slope would have to be justified in each particular situation. In combining the standard deviations, each "sum of squares" had 2 degrees of freedom less than the number of observations obtained from the standard, and 2 less than the number of observations obtained from the unknown.

There were wide differences (from $b=8.36$ to $b=18.73$) among the observed slopes for the individual dosage-response curves in the collaborative study. Variance analysis of the slopes indicated that the differences were significant beyond the 1 per cent level. The slopes from the data of laboratories 1 and 2 were sufficiently higher than the others to suggest their segregation. When this was done, the slopes of the dosage-response curves among laboratories within the two groups agreed within the limits of error. The statistics for standard deviation and for slope were combined for laboratories 1 and 2 and similarly combined for laboratories 3, 4, 5, 6, and 7, and the data of the two groups from the collaborative study recomputed.

Similar analyses of variance showed that no separation was necessary within the second and third series of assays.

In column 4 of Table 4 and columns 3 of Tables 5 and 6 the estimates of potency and their standard errors have been computed for each assay with the combined statistics. The potency at the foot of these columns is the geometric weighted mean.

Exact confidence limits were computed for each assay, using the combined standard deviation and slope from its group. Recently Bliss (19) has discussed the advantages of computing the exact limits. Among these is the adjustment for the sampling error of the combined slope of the dosage-response curves, which error may be relatively large in individual assays based upon few observations. For obtaining exact confidence limits, a modified ts_M' was computed as follows:

$$ts_M' = tC\lambda\sqrt{\frac{1}{N_s}+\frac{1}{N_u}+\frac{(\bar{y}_s-\bar{y}_u)^2}{B^2-s^2t^2}}. \tag{3}$$

In equation (3), t is taken from a table of this statistic (20) with the degrees of freedom for error (s^2) in the group of assays at the level of probability (P) desired. C is a correction factor

$$\text{where } C^2=\frac{B^2}{B^2-s^2t^2}, \text{ see equation 8, (19),}$$

and all the other symbols have the same significance as previously. The term ts_M' is added to and subtracted from the mid-point of the confidence interval to obtain the confidence limits or

$$X_L = \bar{x}_s - \bar{x}_u - \frac{C^2(\bar{y}_s - \bar{y}_u)}{b_c} \pm ts_M'. \qquad (4)$$

In Tables 4, 5, and 6 the confidence limits are given at a probability level corresponding to $P = 0.05$.

The results of the individual assays showed variation in potency, as was to be expected from data of this type. In the collaborative study, the probability of occurrence of the observed variation was about 1 in 5 or well within the sampling error; chi-square = 7.5, $n = 5$. A similar examination of the estimated potencies from the first Du Pont series of assays showed a variation that would be expected to occur by chance only about 35 times in 1000 trials; chi-square = 32.4, $n = 14$. This indicated a significant heterogeneity. By the same test the second Du Pont series of assays showed a very low variation among the potencies of the several assays; chi-square = 1.59, $n = 9$.

RESULTS AND DISCUSSION

We have presented in Tables 4, 5, and 6 the mean values, expressed in A.O.A.C. units per gram, which were obtained from three series of assays for the potency of two different samples of crystalline D_3. The figures for sample 4–188, 54.7 ± 2.82 million from the collaborative assays, and 52.7 ± 2.66 million from the series of assays in this laboratory, agreed well within the sampling error. By combining these mean values sample 4–188 was found to have a potency of 53.7 ± 1.94 million A.O.A.C. units per gram. While the difference between this value and the figure of 58.4 ± 2.75 million for sample 64–122 was not found to be significant statistically ($P = 0.17$) it may, however, express the somewhat greater purity of the latter sample. Alternatively, the difference may be due to the change in technique (the use of toes instead of tibia) in the assays of sample 64–122, but we think this is unlikely. Further assays (using toes) on this sample carried out by us since the above tests were completed confirm the value of approximately 58 million units per gram.

It is to be emphasized that the above values for crystalline D_3 in comparison with reference cod liver oil may apply only to the conditions under which these tests were carried out. There is definite evidence that the diet particularly as regards its content of available phosphorus, has a distinct effect on the efficacy with which different vitamins D bring about deposition of bone salts in certain avian species. Thus Matterson, Scott, and Singsen (22) have recently shown that, for turkey poults, the efficacy of a vitamin D_3 concentrate in comparison with the vitamin D of a cod liver

oil was much higher when the phosphorus of the diet was present mainly as phytin than when it was present in inorganic form. Also Singsen and Mitchell (21) have shown the same relationship to hold for chicks. It is to be remembered that the A.O.A.C. diet, containing 2 per cent of tricalcium phosphate, has a large proportion of its total phosphorus content in available form. Actually, in other experiments (unpublished) we have obtained higher values than those reported above for D_3 in comparison with reference cod liver oil when the chicks were restricted to lower available phosphorus intake.

These findings, together with those that have been published during the last several years on the relative efficacy for poults of different sources of vitamin D including cod liver oil (for literature references see (22)), lead inescapably to the conclusion that cod liver oil either contains a vitamin D different from D_3 or else, and more probably, it contains a multiplicity of antirachitic substances. It thus partakes of the same nature as many other fish oils which were shown to possess different forms of vitamin D (or different proportions of the same substances) a decade or more ago as reported by Bills and his associates (24). In this connection we may state, without further elaboration at this time, that pure vitamin D_3 when compared with reference cod liver oil in this laboratory using *the rat* as test animal yields values much lower than those reported above. This, of course, is the same as saying that the cod liver oil has a higher efficacy relative to D_3 when tested with rats than when tested with chicks and such a situation would be expected if part of the antirachitic activity of cod liver oil were due to D_2 or a vitamin D of similar nature.

We are firmly of the opinion that crystalline vitamin D_3, which now can be prepared in high purity, offers many advantages as an international standard for antirachitic materials. The results presented in this paper should be of value in indicating the quantitative difference which exists between it and reference cod liver oil by the A.O.A.C. chick method of assay.

SUMMARY

1. A collaborative assay of pure vitamin D_3 against reference cod liver oil No. 2 in 7 laboratories, using chicks as the test animals, gave a mean value for the former of 54.7 ± 2.82 million A.O.A.C. units per gram.

2. A series of 16 assays in this laboratory on the same sample of vitamin D_3 as was used in the collaborative study gave a mean value of 52.7 ± 2.66 million A.O.A.C. units per gram. Combining the two mean values for this sample gave an overall mean of 53.7 ± 1.94 million A.O.A.C. units per gram.

3. A series of 11 assays in this laboratory on a second sample of pure vitamin D_3 gave a mean value of 58.4 ± 2.75 million A.O.A.C. units per gram of vitamin D_3.

4. The implications of these findings, and others which have recently been published, as to the nature of the antirachitic substances in cod liver oil have been briefly discussed.

5. Pure crystalline vitamin D_3 is recommended as an international standard for vitamin D.

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NOTE

Suggested Modification of Jones-Robertson Method For Determination of Nitrate Nitrogen In Fertilizers

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The Jones-Robertson method[1] for nitrate nitrogen in fertilizers may fail to give dependable results when applied to certain types of mixed fertilizers, for the following reasons: The 0.5 gram sample may be too small to be representative of heterogeneous materials; and in the presence of reducing matter some of the nitric acid may be reduced either to ammonia, giving high results for residual nitrogen, or else to nitrogen by the ammonium salts present, giving low residual nitrogen results. Since the method determines nitrate nitrogen by difference, low residual nitrogen results mean high nitrate nitrogen results, and vice versa. Experience indicated that the desired reduction to NO proceeds better when hydrochloric acid rather than sulfuric is used in the preliminary boiling, and experiments were devised to confirm this.

A series of tests was run using 0.8 gram of commercial powdered sugar with varying amounts of potassium nitrate. Amounts of nitrate and reducing matter were purposely exaggerated in order to determine their effect more accurately. Results are given in Table 1. The first four tests were run according to the official method; Test No. 5 was first boiled with ferrous sulfate and about 60 ml of 1+1 hydrochloric acid, then the sulfuric acid was added and the digestion continued. Titrations of the distillates from these determinations give a measure of the ammonia produced by reduction of nitrate by sugar.

TABLE 1

TEST NO.	POTASSIUM NITRATE	TITRATION (0.1 *N*)
	gram	*ml*
1	0.2	1.4
2	0.35	5.5
3	0.7	8.2
4	1.0	8.7
5	0.35	0.8

A second series was run using 0.7 gram of commercial tankage ground to about 60 mesh and varying amounts of potassium nitrate, with and without previous boiling with hydrochloric acid, using in all cases 5 grams of $FeSO_4 \cdot 7H_2O$. Results are shown in Table 2 expressed as per cent nitrogen in the tankage. These results are believed to indicate that the nitrate is reduced to ammonia by organic matter, giving high results for residual nitrogen, but that if a large amount of nitrate is present it may oxidize some of the organic or ammoniacal nitrogen to N_2, giving low results. The hydrochloric acid apparently increases the effectiveness of the reduction by iron, thereby eliminating the other effects.

A third series was run to determine the best method of treating with hydrochloric acid. Tests 1–4 were run on 1 gram of sucrose and 0.42 gram of potassium

* Allen B. Lemmon, Chief.

[1] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, sixth edition (1945), 233.

TABLE 2

POTASSIUM NITRATE	ORGANIC N% OFFICIAL METHOD	ERROR	ORGANIC N% PREVIOUS BOILING WITH HCl	ERROR
gram				
0	12.69*	—	12.69	—
0.1	12.88	+.19	12.72	+.03
0.2	12.96	+.27	12.71	+.02
0.3	12.97	+.28	12.61	−.08
0.4	12.92	+.23	—	—
0.5	12.83	+.14	12.64	−.05
0.6	11.00	−1.69	12.60	−.09

* Average of two determinations: 12.68, 12.71.

nitrate in a total of 20 ml soln; Test 5 was on the same weights of material in the dry state. In all tests 5 grams of $FeSO_4 \cdot 7H_2O$ were added, followed by water and acid as indicated in Table 3. Titrations were with 0.2 *N* acid.

TABLE 3.—*Comparison of various methods of treatment*

TEST NO.	TREATMENT	TITRATION
		ml
1	+40 ml water+30 ml sulfuric acid	5.52
2	+30 ml water+30 ml hydrochloric acid, boil, +30 ml sulfuric acid	0.53
3	+30 ml water+30 ml hydrochloric acid+30 ml sulfuric acid	0.46
4	+90 ml soln of equal volumes of water, sulfuric acid, and hydrochloric acid	0.32
5	(Same as sample 4)	0.24

The final modification suggested is as follows:

Weigh out 1.4 gram if not over 10% total N, or 0.7 gram if over 10%. Add 5 grams $FeSO_4 \cdot 7H_2O$ and 90 ml of a soln containing equal volumes of water, sulfuric acid, and hydrochloric acid. Boil down to fumes and continue as in Official Method.[1] To prepare the acid soln, mix the water and sulfuric acid in advance and add hydrochloric acid after cooling.

Acknowledgment is made to Leslie Titus, formerly of this Bureau, for suggesting the use of hydrochloric acid in this method.

BOOK REVIEW

Extractives from Northeastern Woods Bulletin No. 9, August, 1946. Issued by Northeastern Wood Utilization Council, P. O. Box 1577, New Haven 6, Conn. 62 pages. Price $1.00.

The bulletin contains a note, "America's Need for Essential Oils," by Percy C. Magnus, two articles, "Extractives, a Review of Literature" and "The Economic Extraction of Essential Oils" by Robert S. Aries; an article "Commercial Production in the Northeast" by Arthur H. Downey; a bibliography on the conifer leaf industry; a table of recent essential oil imports into the United States, and a list of American firms producing or dealing with essential oils. Yields, apparatus, capital cost, and operating budget for a small extraction plant are described. The bulletin assembles in convenient form general information regarding essential oils and other extractives, which should be useful to interested regulatory agencies, and to present and prospective manufacturers of these products.

J. W. SALE