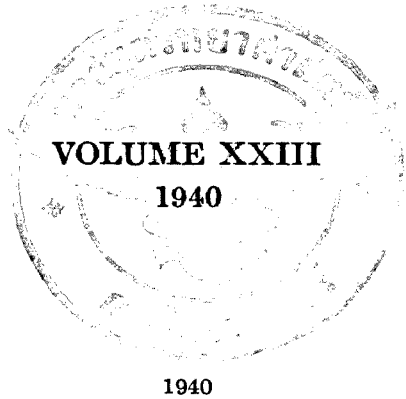
JOURNAL

OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS



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BURT LAWS HARTWELL, 1865–1939

BURT LAWS HARTWELL

The subject of this sketch, Burt Laws Hartwell, the son of a farmer, was born in Littleton, Massachusetts, December 18, 1865.

He became a member of the Freshman Class at the Massachusetts Agricultural College, now Massachusetts State College, in the autumn of 1885, and was graduated four years later with the degree of B.Sc. Recognizing the importance of its cultural activities, he joined the College Shakespearean Club, of which he was an active and influential member throughout his college course. He also took a prominent part in the student debating (Washington Irving) society, and in his freshman year won the Farmswourth-Kendall Gold Medal for excellence in declamation.

Upon graduation from college, Hartwell became Assistant Chemist in the Massachusetts Agricultural Experiment Station, under the leadership of his Professor of Chemistry, Professor Charles A. Goessmann, and in the autumn of 1891 he accepted a like position at the Rhode Island Experiment Station. On September 9, of the same year, he was united in marriage with Miss May Louise Smith, of Stowe, Massachusetts, who survives him.

For a long time Hartwell had desired to study further and secure an advanced degree, but to accomplish this there was required a knowledge of German in addition to French, which he had taken in college. With his characteristic energy and tenacity he took up the study of German, and in a year or two, with the occasional aid of the writer, was able to meet the University requirements.

Hartwell then asked for an academic year's leave of absence, which the Board of Managers of the Rhode Island State College and Experiment Station granted, and later extended for another year. This evidenced the Board's appreciation of him, and their desire to retain his services. In June, 1903, the University of Pennsylvania conferred upon him the degree of Ph.D.

When, in 1902, the Chief Chemist was promoted to the Directorship, Hartwell was slated to succeed him, and in 1912, when the Director resigned to enter commercial work, Hartwell was made Director. He had already taught Agricultural Chemistry for several years in the Rhode Island State College. Hartwell filled the position of Director with marked ability, but resigned in 1928 due to differences of opinion regarding Station policy. Immediately the *Providence Journal*, which had staunchly supported him, invited him to become their Agricultural Columnist, a position which he held until his death, on July 12, 1939.

All his life Hartwell was interested in music, having served as tenor in the college choir, also in that of the Kingston Village Church, and up to the time of his death he was a member of the quartette of the Washington Park Men's Club.

In his earlier years Hartwell was an ardent tennis player, and gardening was his life-long hobby. For practically all his adult life he was a member of the order of Patrons of Husbandry and when, on one occasion, the finances of the State Grange became somewhat entangled Hartwell, in recognition of his ability and integrity, was the man selected to untangle them.

Hartwell served in 1907 as a member of the Executive Committee of the Association of Official Agricultural Chemists, and as Chairman of the Crop Protection Institute of the National Research Council assisted in presenting to the Association the results of that Committee's work.

It was a source of great satisfaction to Dr. Hartwell in June, 1929, that he was able to participate with his surviving classmates in the celebration of the fiftieth anniversary of his graduation from college.

Some idea of Dr. Hartwell's scientific contributions may be gained from the fact that he was author or joint author of no less than 143 articles published in the Experiment Station bulletins and various scientific journals, not to mention his con-

tributions to the Annual Reports of the Station—an average of four or more papers for every year that he served Rhode Island. These papers covered a wide range of subjects, including a study of the composition of Rhode Island sea-weeds and soils, soil acidity, the effect of chlorides, sulfur, lime, magnesia, soda, manganese, aluminum salts, and the various sources of nitrogen, phosphorus, and potassium for plant growth. Among other notable studies were those on crop rotation, the effect of certain plants upon the growth of those that follow, and the study of the effect of green manures, animal manures, and fertilizers upon the growth of truck crops.

During his lifetime Dr. Hartwell made a host of friends, who admired him for his good judgment, sound reasoning, dry humor, true friendship, his thorough dependence as a scientist, and for his studied attempts to do something of real value to the American farmer and mankind. Rhode Island will never forget his service to the State!—HOMER J. WHEELER.

PROCEEDINGS OF THE FIFTY-FIFTH ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1939

The fifty-fifth annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington, D. C., October 30, 31 and November 1, 1939.

The meeting was called to order by the president, W. S. Frisbie, U. S. Food and Drug Administration, Washington, D. C., on the morning of October 30, at 10:30 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING NOVEMBER, 1940

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G. G. FRARY, Vermillion, S. D.
J. O. CLARKE, Chicago, Ill.
W. S. FRISBIE, Washington, D. C.

PERMANENT COMMITTEES

Recommendations of Referees

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

H. A. LEPPER (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*

SUBCOMMITTEE A: G. E. GRATTAN (1940) (Department of Agriculture, Ottawa, Can.), *Chairman;* H. A. HALVORSON (1942) and E. L. GRIFFIN (1944). [Standard solutions (silver nitrate and thiocyanate, iodine, sodium thiosulfate, sulfuric acid, arsenite, constant boiling hydrochloric acid); insecticides, fungicides, and caustic poisons (fluorine compounds; pyrethrins, derris, and cubé; naphthalene in poultry lice products); soils and liming materials (hydrogen-ion concentration—soils of arid and semi-arid regions and soils of humid regions; liming materials, less common metals in soils); feeding stuffs (sampling, ash, mineral mixed feeds—calcium and iodine; lactose in mixed feeds, hydrocyanic acid in glucoside-bearing materials, vitamin D for poultry, carotene, manganese, adulteration of condensed milk products and of cod-liver oil, fat in fish meal); fertilizers (phosphoric acid, nitrogen, magnesium and manganese, acid- and base-forming quality, potash; calcium and sulfur, copper and zinc); plants

(iodine, carbodydrates, hydrocyanic acid, zinc, copper and cobalt, boron, chlorophyl and carotene); lignin, enzymes (papain); paints, paint materials and varnishes (accelerated testing of paints, varnishes); vitamins (vitamin A, vitamin B, vitamin C, vitamin D, vitamin K, riboflavin); leathers and tanning materials.]

SUBCOMMITTEE B: H. J. FISHER (1940) (Agricultural Experiment Station, New Haven, Conn.), *Chairman;* A. E. PAUL (1942) and W. F. REINDOLLAR (1944). [Naval stores; radioactivity; cosmetics (hair preparations, colored make-up preparations, facial preparations, dentifrices and mouth washes, miscellaneous); coloring matters in drugs and cosmetics (non-pigment colors, lakes and pigments, analysis of color mixtures, identification of coal-tar colors, tests for coal-tar color intermediates, spectrophotometric color testing, micro methods for coal-tar colors); synthetic drugs (benzedrine, hydroxyquinoline sulfate, methylene blue; aminopyrine, acetophenetidin, and caffeine; ethyl aminobenzoate, sulfapyridine); vegetable drugs and their derivatives (chemical methods for ergot alkaloids, theophyllin sodium salicylate, physostigmine salicylate, arecoline hydrobromide, quinine ethyl carbonate, theobromine and phenobarbital, plasmochine); drug bioassays; miscellaneous drugs (microchemical tests for alkaloids and synthetics, iodine ointment, magnesium trisilicate, mercury ointment, emulsions, compound ointment of benzoic acid, elixir of three bromides).]

SUBCOMMITTEE C: G. G. FRARY (1940) (State Chemical Laboratory, Vermillion, S. D.), *Chairman;* W. B. WHITE (1942) and J. O. CLARKE (1944). [Dairy products (Babcock method, butter, cheese—isolation of fat, malted milk—fat, casein, dried milk—lactic acid, lactose in milk, mold in butter, decomposition, neutralizers, difference between dairy products made from cows' milk and those made from milk of other animals, frozen desserts, tests for pasteurization—milk and cream, butter, unification of methods for ash in milk and evaporated milk, sugar in sweetened condensed milk); oils, fats and waxes (refractometric determination of oils in seeds, thiocyanogen number, Polenske number); eggs and egg products (unsaponifiable constituents and fat, detection of decomposition, added glycerol); metals in foods (arsenic and antimony, copper, zinc, fluorine, lead, mercury, selenium, fumigation residues in foods); canned foods (tomato products); meats and meat products; spices and condiments (salad dressings, vinegar, volatile constituents, mustard and mustard products); gums in foods (starchy foods); microbiological methods (canned fish products, canned meats, canned vegetables, canned fruit products, sugar, eggs and egg products); fish and other marine products (solids and fats), coffee and tea, food preservatives—saccharin (benzoate of soda); coloring matters in foods, microchemical methods.]

SUBCOMMITTEE D: W. C. JONES (1940) (Department of Agriculture, Richmond, Va.), *Chairman;* J. W. SALE (1942) and J. A. LECLERC (1944). [Sugars and sugar products (acetyl-methyl carbinol and diacetyl in food products, sucrose in molasses, honey, refractive indices of sugar solutions, maple products; drying, densimetric, and refractometric methods; polariscopic methods, chemical methods for reducing sugars, unfermented reducing substances in molasses); waters, brine, and salt (moisture in effervescent salts); alcoholic beverages (diastatic activity of malt, proteolytic activity of malt, malt extract in malt, malt adjuncts, beer, CO_2 in beer, heavy metals in beer, hops, volatile acids in wine, SO_2 in wine and beer, whiskey and rum, detection of adulteration of distilled spirits, wood alcohol in distilled spirits, denaturants in distilled spirits,

cordials and liqueurs); fruits and fruit products (electrometric titration of acids; malic, isocitric, citric, and lactic acids; polariscopic methods for jams, jellies, and preserves; Na and Cl in jams, jellies and other fruit products; P_2O_5 in jams, jellies, and other fruit products—colorimetric and gravimetric; K_2O in jams, jellies and other fruit products); cacao products, milk protein, chocolate in sweet and milk chocolate, lecithin; cereal foods (macaroni products; H-ion concentration, starch in raw and cooked cereals, acidity of fat of flour, grain and cornmeal, sugar in flour, baking test for soft wheat flour, flour-bleaching chemicals, CO_2 in self-rising flour, milk solids in milk bread, cold water extract flour, proteolytic enzymes, color of flour and bread, soya flour in foods, whole wheat flour, phosphated flour, sterols, corn products, oat products, rye and buckwheat, barley and rice, baked products other than bread, moisture in self-rising and pancake flours, etc.); flavors and non-alcoholic beverages (organic solvents in flavors, glycerol, vanillin, and coumarin).]

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STANDARD SOLUTIONS:

General referee: R. L. Vandaveer, Food and Drug Adm., New Orleans, La.

SODIUM THIOSULFATE:

Associate referee: R. L. Vandaveer.

PREPARATION OF CONSTANT BOILING HCl:

Associate referee: R. L. Vandaveer.

SILVER NITRATE AND THIOCYANATE:

Associate referee: E. C. Deal, Food and Drug Adm., New Orleans, La.

IODINE:

Associate referee: George M. Johnson, Food and Drug Adm., St. Louis, Mo.

SULFURIC ACID:

Associate referee: Harry Conroy, Food and Drug Adm., Kansas City, Mo.

ARSENITE:

Associate referee: George M. Johnson.

INSECTICIDES, FUNGICIDES, AND CAUSTIC POSIONS:

General referee: J. J. T. Graham, Food and Drug Adm., Washington, D. C.

PYRETHRINS, DERRIS, AND CUBE:

Associate referee: J. J. T. Graham.

FLUORINE COMPOUNDS:

Associate referee: C. G. Donovan, Food and Drug Adm., Washington, D. C.

NAPHTHALENE IN POULTRY LICE PRODUCTS:

Associate referee: Roswell Jinkins, Food and Drug Adm., Chicago, Ill.

SOILS AND LIMING MATERIALS:

General referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

HYDROGEN-ION CONCENTRATION:

a. SOILS OF ARID AND SEMI-ARID REGIONS:

Associate referee: W. T. McGeorge, Agricultural Experiment Station, Tucson, Ariz.

b. SOILS OF HUMID REGIONS:

Associate referee: E. R. Purvis, Virginia Truck Experiment Station, Norfolk, Va.

LESS COMMON METALS IN SOILS:

Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

LIMING MATERIALS:

Associate referee: W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

FEEDING STUFFS:

General referee: L. S. Walker, Agricultural Experiment Station, Burlington, Vt.

SAMPLING:

Associate referee: L. M. Jeffers, Dept. of Agriculture, Sacramento, Calif.

ASH:

Associate referee: J. L. St. John, Agricultural Experiment Station, Pullman, Wash.

MINERAL MIXED FEEDS (calcium and iodine):

Associate referee: A. T. Perkins, Kansas State College, Manhattan, Kan.

LACTOSE IN MIXED FEEDS:

Associate referee: D. A. Magraw, American Dry Milk Inst., Chicago, Ill.

HYDROCYANIC ACID IN GLUCOSIDE-BEARING MATERIALS:

Associate referee: R. A. Greene, University of Arizona, Tucson, Ariz.

VITAMIN D FOR POULTRY:

Associate referee: C. D. Tolle, Food and Drug Adm., Washington, D. C.

MANGANESE:

Associate referee: J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

CAROTENE:

Associate referee: A. R. Kemmerer, Agricultural Experiment Station, College Station, Tex.

FAT IN FISH MEAL:

Associate referee: R. W. Harrison, Bureau of Fisheries, Seattle, Wash.

ADULTERATION OF CONDENSED MILK PRODUCTS AND COD-LIVER OIL:

Associate referee: P. B. Curtis, Agricultural Experiment Station, Lafayette Ind.

FERTILIZERS:

General referee: G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

PHOSPHORIC ACID:

Associate referee: W. H. Ross, Bureau of Chemistry and Soils, Washington, D. C.

NITROGEN:

Associate referee: A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

MAGNESIUM AND MANGANESE:

Associate referee: J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

POTASH:

Associate referee: O. W. Ford, Agricultural Experiment Station, Lafayette, Ind.

ACID AND BASE-FORMING QUALITY:

Associate referee: H. R. Allen, Agricultural Experiment Station, Lexington, Ky.

CALCIUM AND SULFUR:

Associate referee: Gordon Hart, Department of Agriculture, Tallahassee, Fla.

COPPER AND ZINC:

Associate referee: W. Y. Gary, Department of Agriculture, Tallahassee, Fla.

PLANTS:

General referee: E. J. Miller, Agricultural Experiment Station, E. Lansing, Mich.

IODINE:

Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

CARBOHYDRATES:

Associate referee: J. T. Sullivan, U. S. Regional Pasture Research Lab., State College, Pa.

ZINC:

Associate referee: Hale Cowling, Agricultural Experiment Station, East Lansing, Mich.

COPPER AND COBALT:

Associate referee: Lillian I. Butler, Agricultural Experiment Station, East Lansing, Mich.

BORON:

Associate referee: R. L. Cook, Agricultural Experiment Station, East Lansing, Mich.

CHLOROPHYL AND CAROTENE:

Associate referee: E. J. Benne, Agricultural Experiment Station, East Lansing, Mich.

HYDROCYANIC ACID:

Associate referee: R. A. Greene, University of Arizona, Tucson, Ariz.

LIGNIN:

General referee: M. Phillips, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

ENZYMES (papain):

General referee: R. R. Thompson, Hawaiian Agricultural Station, Honolulu, Hawaii.

PAINTS, PAINT MATERIALS AND VARNISHES:

General referee: C. S. Ladd, Food Commissioner and Chemist, Bismarck, N. D.

ACCELERATING TESTING OF PAINTS:

Associate referee: L. L. Carrick, Agricultural Experiment Station, Fargo, N. D.

VARNISHES:

Associate referee: F. Roberts, Paint and Varnish Lab., Bismarck, N. D.

VITAMINS:

General referee: E. M. Nelson, Food and Drug Adm., Washington, D. C.

VITAMIN A:

Associate referee: J. B. Wilkie, Food and Drug Adm., Washington, D. C.

VITAMIN B_1:

Associate referee: O. L. Kline, Food and Drug Adm., Washington, D. C.

VITAMIN C:

Associate referee: Otto A. Bessey, Harvard Medical School, Boston, Mass.

VITAMIN D:

Associate referee: W. C. Russell, Agricultural Experiment Station, New Brunswick, N. J.

VITAMIN K:

Associate referee: H. J. Almquist, University of California, Berkeley, Calif.

RIBOFLAVIN:

Associate referee: A. R. Kemmerer, Agricultural Experiment Station, College Station, Texas.

LEATHERS AND TANNING MATERIALS:

General referee: I. D. Clarke, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

NAVAL STORES:

General referee: V. E. Grotlisch, Food and Drug Adm., Washington, D. C.

RADIOACTIVITY:

General referee: A. Wolf, Food and Drug Adm., Washington, D. C.

COSMETICS:

General referee: Dan Dahle, Food and Drug Adm., Washington, D. C.

HAIR PREPARATIONS:

Associate referee: I. S. Shupe, Food and Drug Adm., Washington, D. C.

COLORED MAKE-UP PREPARATIONS:
Associate referee: E. M. Hoshall, Food and Drug Adm., Baltimore, Md.

FACIAL PREPARATIONS:
Associate referee: C. F. Bruening, Food and Drug Adm., Baltimore, Md.

DENTIFRICES and MOUTH WASHES:
Associate referee: E. J. Hennessy, State Labs., Bismarck, N. D.

MISCELLANEOUS:
Associate referee: E. W. Campbell, State House, Augusta, Me.

COLORING MATTERS IN DRUGS AND COSMETICS:
General referee: V. E. Grotlisch, Food and Drug Adm., Washington, D. C.

NON-PIGMENT COLORS:
Associate referee: S. S. Forrest, Food and Drug Adm., Washington, D. C.

LAKES AND PIGMENTS:
Associate referee: G. R. Clark, Food and Drug Adm., Washington, D. C.

ANALYSIS OF COLOR MIXTURES:
Associate referee: O. L. Evenson, Food and Drug Adm., Washington, D. C.

IDENTIFICATION OF COAL-TAR COLORS:
Associate referee: W. H. King, Food and Drug Adm., New Orleans, La.

TESTS FOR COAL-TAR COLOR INTERMEDIATES:
Associate referee: S. H. Newburger, Food and Drug Adm., Washington, D. C.

SPECTROPHOTOMETRIC COLOR TESTING:
Associate referee: R. W. Stewart, Food and Drug Adm., Washington, D. C.

MICRO METHODS FOR COAL-TAR COLOR ANALYSIS:
Associate referee: J. A. Kime, Food and Drug Adm., Washington, D. C.

SYNTHETIC DRUGS:
General referee: L. E. Warren, Food and Drug Adm., Washington, D. C.

BENZEDRINE:
Associate referee: J. H. Cannon, Food and Drug Adm., St. Louis, Mo.

HYDROXYQUINOLINE SULFATE:
Associate referee: W. H. Hartung, University of Maryland, Baltimore, Md.

METHYLENE BLUE:
Associate referee: H. O. Moraw, Food and Drug Adm., Chicago, Ill.

AMINOPYRINE, ACETOPHENETIDIN, AND CAFFEINE:
Associate referee: Jonas Carol, Food and Drug Adm., Cincinnati, Ohio.

ETHYL AMINOBENZOATE:
Associate referee: J. R. Matchett, Bureau of Internal Revenue, Washington, D. C.

SULFAPYRIDINE:
Associate referee: I. Schurman, Food and Drug Adm., Chicago, Ill.

VEGETABLE DRUGS AND THEIR DERIVATIVES:
General Referee: F. H. Wiley, Food and Drug Adm., Washington, D. C.

CHEMICAL METHODS FOR ERGOT ALKALOIDS:
Associate referee: D. C. Grove, Food and Drug Adm., Washington, D. C.

THEOPHYLLIN SODIUM SALICYLATE:
Associate referee: M. Harris, Food and Drug Adm., Houston, Tex.

PHYSOSTIGMINE SALICYLATE:
Associate referee: G. M. Johnson, Food and Drug Adm., St. Louis, Mo.

ARECOLINE HYDROBROMIDE:
Associate referee: H. R. Bond, Food and Drug Adm., Kansas City, Mo.

QUININE ETHYL CARBONATE:
Associate referee: H. G. Underwood, Food and Drug Adm., Chicago, Ill.

THEOBROMINE AND PHENOBARBITAL:
Associate referee: E. C. Deal, Food and Drug Adm., New Orleans, La.

PLASMOCHINE:
Associate referee: F. C. Sinton, Food and Drug Adm., New York City.

DRUG BIOASSAYS:
General referee: L. C. Miller, Food and Drug Adm., Washington, D. C.

MISCELLANEOUS DRUGS:
General referee: C. K. Glycart, Food and Drug Adm., Chicago, Ill.

MICROCHEMICAL TESTS FOR ALKALOIDS AND SYNTHETICS:
Associate referee: G. L. Keenan, Food and Drug Adm., Washington, D. C.

IODINE OINTMENT:
Associate referee: W. F. Reindollar, State Dept. of Health, Baltimore, Md.

MAGNESIUM TRISILICATE:
Associate referee: E. K. Tucker, Dept. Agriculture and Industry, Montgomery, Ala.

MERCURY OINTMENT:
Associate referee: P. S. Jorgensen, Food and Drug Adm., San Francisco, Calif.

EMULSIONS:
Associate referee: E. C. Payne, Food and Drug Adm., Chicago, Ill.

COMPOUND OINTMENT OF BENZOIC ACID:
Associate referee: W. F. Kunke, Food and Drug Adm., Chicago, Ill.

ELIXIR OF THREE BROMIDES:
Associate referee: R. Hyatt, Food and Drug Adm., Cincinnati, Ohio.

DAIRY PRODUCTS:

General referee: G. G. Frary, Dairy and Food Dept., Vermillion, S. D.

BUTTER:

Associate referee: J. A. Mathews, Food and Drug Adm., Washington, D. C.

CHEESE (isolation of fat):

Associate referee: I. D. Garard, Rutgers University, New Brunswick, N. J.

MALTED MILK (fat):

Associate referee: E. W. Coulter, Food and Drug Adm., Chicago, Ill.

MALTED MILK (casein):

Associate referee: I. Schurman, Food and Drug Adm., Chicago, Ill.

DRIED MILK (lactic acid):

Associate referee: F. Hillig, Food and Drug Adm., Washington, D. C.

FROZEN DESSERTS:

Associate referee: F. Leslie Hart, Food and Drug Adm., Los Angeles, Calif.

LACTOSE IN MILK:

Associate referee: E. R. Garrison, University of Missouri, Columbia, Mo.

MOLD IN BUTTER:

Associate referee: J. D. Wildman, Food and Drug Adm., Washington, D. C.

TESTS FOR PASTEURIZATION OF MILK AND CREAM:

Associate referee: F. W. Gilcreas, Department of Health, Albany, N. Y.

TESTS FOR PASTEURIZATION OF BUTTER:

Associate referee: P. H. Brewer, Purdue University, Lafayette, Ind.

DIFFERENCE BETWEEN DAIRY PRODUCTS MADE FROM COWS' MILK AND THOSE MADE FROM THE MILK OF OTHER ANIMALS:

Associate referee: I. D. Garard.

DECOMPOSITION IN DAIRY PRODUCTS:

Associate referee: C. S. Myers, Food and Drug Adm., Washington, D. C.

SUGARS IN SWEETENED CONDENSED MILK:

Associate referee: J. B. Snider, Food and Drug Adm., Minneapolis, Minn.

NEUTRALIZERS IN DAIRY PRODUCTS:

Associate referee: F. Hillig.

UNIFICATION OF METHODS FOR ASH IN MILK AND EVAPORATED MILK:

Associate referee: Burton Jordon, Dairy and Food Dept., Vermillion, S. D.

OILS, FATS AND WAXES:

General referee: J. Fitelson, Food and Drug Adm., New York, N. Y.

REFRACTOMETRIC DETERMINATION OF OIL IN SEEDS:

Associate referee: Lawrence Zeleny, Bureau of Agricultural Economics, Washington, D. C.

THIOCYANOGEN NUMBER:

Associate referee: H. P. Trevithick, New York Produce Exchange, New York City.

POLENSKE NUMBER:

Associate referee: J. A. Mathews, Food and Drug Adm., Washington, D. C.

EGGS AND EGG PRODUCTS:

General referee: E. O. Haenni, Food and Drug Adm., Washington, D. C.

UNSAPONIFIABLE CONSTITUTENTS AND FAT:

Associate referee: E. O. Haenni.

DETECTION OF DECOMPOSITION:

Associate referee: L. C. Mitchell, Food and Drug Adm., St. Louis, Mo.

ADDED GLYCEROL:

Associate referee: L. C. Mitchell.

METALS IN FOODS:

General referee: H. J. Wichmann, Food and Drug Adm., Washington, D. C.

ARSENIC AND ANTIMONY:

Associate referee: C. C. Cassil, Bureau of Entomology and Plant Quarantine, Washington, D. C.

COPPER:

Associate referee: A. C. Greenleaf, National Canners Assn., Washington, D. C.

ZINC:

Associate referee: W. S. Ritchie, Agricultural Experiment Station, Amherst, Mass.

FLUORINE:

Associate referee: P. A. Clifford, Food and Drug Adm., Washington, D. C.

LEAD:

Associate referee: P. A. Clifford.

MERCURY

Associate referee: W. O. Winkler, Food and Drug Adm., Washington, D. C.

SELENIUM:

Associate referee: A. K. Kline, Food and Drug Adm., San Francisco, Calif.

FUMIGATION RESIDUES IN FOODS:

Associate referee: W. O. Winkler.

COLORING MATTERS IN FOODS:

General referee: C. F. Jablonski, Food and Drug Adm., New York City.

FRUITS AND FRUIT PRODUCTS:

General referee: B. G. Hartmann, Food and Drug Adm., Washington, D. C.

ELECTROMETRIC TITRATION OF ACIDS:

Associate referee: R. U. Bonnar, Food and Drug Adm., Washington, D. C.

MALIC, ISOCITRIC, CITRIC, AND LACTIC ACIDS:
Associate referee: B. G. Hartmann.

K_2O IN JAMS, JELLIES, AND OTHER FRUIT PRODUCTS:
Associate referee: C. A. Wood, Food and Drug Adm., New York City.

P_2O_5 IN JAMS, JELLIES, AND OTHER FRUIT PRODUCTS:
Associate referees: Colorimetric—H. W. Gerritz, Food and Drug Adm., San Francisco, Calif.; Gravimetric—H. Shuman, Food and Drug Adm., Philadelphia, Pa.

POLARISCOPIC METHODS FOR JAMS, JELLIES, AND PRESERVES:
Associate referee: R. A. Osborn, Food and Drug Adm., Washington, D. C.

Na AND Cl IN JAMS, JELLIES, AND OTHER FRUIT PRODUCTS:
Associate referee: R. S. Pruitt, Food and Drug Adm., Cincinnati, Ohio.

CANNED FOODS:
General referee: V. B. Bonney, Food and Drug Adm., Washington, D. C.

TOMATO PRODUCTS:
Associate referee: L. M. Beacham, Jr., Food and Drug Adm., Washington, D. C.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:
General referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

ORGANIC SOLVENTS IN FLAVORS:
Associate referee: R. D. Stanley, Food and Drug Adm., Chicago, Ill.

GLYCEROL, VANILLIN, AND COUMARIN:
Associate referee: Llewelyn Jones, Food and Drug. Adm., Kansas City, Mo.

MEATS AND MEAT PRODUCTS:
General referee: R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

CACAO PRODUCTS:
General referee: W. O. Winkler, Food and Drug Adm., Washington, D. C.

MILK PROTEIN:
Associate referee: M. L. Offutt, Food and Drug Adm., New York City.

LECITHIN:
Associate referee: J. H. Bornmann, Food and Drug Adm., Chicago, Ill.

CHOCOLATE IN SWEET AND MILK CHOCOLATE:
Associate referee: W. O. Winkler.

GUMS IN FOODS:
General referee: F. Leslie Hart, Food and Drug Adm., Los Angeles, Calif.

STARCHY FOODS:
Associate referee: D. A. Ballard, Food and Drug Adm., San Francisco, Calif.

SPICES AND CONDIMENTS:
General referee: S. Alfend, Food and Drug Adm., St. Louis, Mo.

VOLATILE CONSTITUENTS:

Associate referee: J. F. Clevenger, Food and Drug Adm., New York City.

VINEGAR:

Associate referee: A. M. Henry, Food and Drug Adm., Atlanta, Ga.

SALAD DRESSINGS:

Associate referee: L. T. Ryan, Regulatory Laboratory, Bismarck, N. D.

MUSTARD AND MUSTARD PRODUCTS:

Associate referee: Tom Field, Food and Drug Adm., St. Louis, Mo.

MICROBIOLOGICAL METHODS:

General referee: A. C. Hunter, Food and Drug Adm., Washington, D. C.

CANNED FISH PRODUCTS:

Associate referee: O. W. Lang, Hooper Foundation Medical Research, University of California, San Francisco, Calif.

CANNED MEATS:

Associate referee: M. L. Laing, Armour and Company, Chicago, Ill.

CANNED VEGETABLES:

Associate referee: E. J. Cameron, National Canners Assn., Washington, D. C.

CANNED FRUITS:

Associate referee: B. A. Linden, Food and Drug Adm., Washington, D. C.

SUGAR:

Associate referee: E. J. Cameron.

EGGS AND EGG PRODUCTS:

Associate referee: Roy Schneiter, Food and Drug Adm., Washington, D. C.

FISH AND OTHER MARINE PRODUCTS:

General referee: H. D. Grigsby, Food and Drug Adm., Philadelphia, Pa.

SOLIDS AND FATS:

Associate referee: Manuel Tubis, Food and Drug Adm., Philadelphia, Pa.

COFFEE AND TEA:

Associate referee: H. J. Fisher, Agricultural Experiment Station, New Haven, Conn.

SUGARS AND SUGAR PRODUCTS:

General referee: R. F. Jackson, National Bureau of Standards, Washington, D. C.

ACETYL-METHYL CARBINOL AND DIACETYL IN FOOD PRODUCTS:

Associate referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

UNFERMENTED REDUCING SUBSTANCES IN MOLASSES:

Associate referee: F. W. Zerban, Sugar Trade Labr., New York City.

SUCROSE IN MOLASSES:

Associate referee: R. A. Osborn, Food and Drug Adm., Washington, D. C.

HONEY:

Associate referee: R. A. Osborn.

MAPLE PRODUCTS:

Associate referee: J. L. Perlman, Dept. of Agriculture and Markets, Albany, N. Y.

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS:

Associate referee: C. F. Snyder, National Bureau of Standards, Washington, D. C.

POLARISCOPIC METHODS (GENERAL):

Associate referee: R. M. Kingsbury, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

CHEMICAL METHODS FOR REDUCING SUGARS:

Associate referee: R. F. Jackson.

REFRACTIVE INDICES OF SUGAR SOLUTIONS:

Associate referee: C. F. Snyder

WATERS, BRINE, AND SALT:

General referee: A. E. Mix, Food and Drug Adm., Washington, D. C.

MOISTURE IN EFFERVESCENT SALTS:

Associate referee: A. E. Mix.

CEREAL FOODS:

General referee: V. E. Munsey, Food and Drug Adm., Washington, D. C.

MACARONI PRODUCTS:

Associate referee: R. H. Harris, Agricultural Experiment Station, Fargo, N. D.

H-ION CONCENTRATION:

Associate referee: George Garnatz, The Kroger Food Foundation, Cincinnati, Ohio.

ACIDITY OF FAT OF FLOUR, GRAIN, AND CORN MEAL:

Associate referee: Lawrence Zeleny, Bureau of Agricultural Economics, Washington, D. C.

STARCH IN RAW AND COOKED CEREALS:

Associate referee: C. Y. Hopkins, National Research Council, Ottawa, Can.

SUGAR IN FLOUR:

Associate referee: R. M. Sanstedt, Agricultural Experiment Station, Lincoln, Neb.

BAKING TEST FOR SOFT WHEAT FLOUR:

Associate referee: E. G. Bayfield, Agricultural Experiment Station, Lincoln, Neb.

FLOUR-BLEACHING CHEMICALS:

Associate referee: Dorothy Scott, Food and Drug Adm., New York City.

CO_2 IN SELF-RISING FLOUR:

Associate referee: Rufus A. Barackman, Victor Chemical Works, Chicago Heights, Ill.

MILK SOLIDS IN MILK BREAD:

Associate referee: V. E. Munsey.

COLD WATER EXTRACT FLOUR:

Associate referee: H. C. Fellows, Bureau of Agricultural Economics, Washington, D. C.

PROTEOLYTIC ENZYMES:

Associate referee: Quick Landis, Fleischmann Labs., New York City.

COLOR OF FLOUR AND BREAD:

Associate referee: H. K. Parker, Novadel-Agene Corporation, Newark, N. J.

SOYA FLOUR IN FOODS:

Associate referee: J. W. Hayward, Archer-Daniels-Midland Co., Milwaukee, Wis.

WHOLE WHEAT FLOUR:

Associate referee: E. J. Hennessy, N. Dakota Reg. Dept., Bismarck, N. D.

PHOSPHATED FLOUR:

Associate referee: J. R. Davies, General Foods Corp., Hoboken, N. J.

STEROLS:

Associate referee: E. O. Haenni, Food and Drug Adm., Washington, D. C.

CORN PRODUCTS:

Associate referee: L. R. Brown, A. E. Staley Mfg. Co., Decatur, Ill.

OAT PRODUCTS:

Associate referee: H. P. Howells, Quaker Oats Co., Cedar Rapids, Iowa.

RYE AND BUCKWHEAT:

Associate referee: C. G. Harrel, Pillsbury Flour Mills Co., Minneapolis, Minn.

BARLEY AND RICE:

Associate referee: Allen D. Dickson, Bureau of Plant Industry, Madison, Wis.

BAKED PRODUCTS OTHER THAN BREAD:

Associate referee: S. Voris, Loose-Wiles Biscuit Co., Long Island City, N. Y.

MOISTURE IN SELF-RISING AND PANCAKE FLOURS, ETC.

Associate referee: L. H. Bailey, Bur. of Agricultural Chemistry and Engineering, Washington, D. C.

MICROCHEMICAL METHODS:

Associate referee: E. P. Clark, Bureau of Entomology and Plant Quarantine, Washington, D. C.

ALCOHOLIC BEVERAGES:

General referee: J. W. Sale, Food and Drug Adm., Washington, D. C.

DIASTATIC ACTIVITY OF MALT:

Associate referee: Christian Rask, Albert Schwill Co., Chicago, Ill.

PROTEOLYTIC ACTIVITY OF MALT:

Associate referee: Stephen Laufer, Schwarz Laboratories, Inc., New York City.

HEAVY METALS IN BEER:

Associate referee: W. H. Harrison, Continental Can Co., Chicago, Ill.

CARBON DIOXIDE IN BEER:

Associate referee: P. P. Gray, Wallerstein Laboratories, New York City.

MALT EXTRACT IN MALT:

Associate referee: E. A. Siebel, 8 S. Dearborn St., Chicago, Ill.

MALT ADJUNCTS:

Associate referee: F. P. Siebel, Siebel Institute, Chicago, Ill.

BEER:

Associate referee: H. W. Rohde, Schlitz Brewing Co., Milwaukee, Wis.

HOPS:

Associate referee: Frank Robak, Bureau of Plant Industry, Washington, D. C.

VOLATILE ACIDS IN WINES:

Associate referee: M. A. Joslyn, Agricultural Experiment Station, Berkeley Calif.

SULFUR DIOXIDE IN BEER AND WINE:

Associate referee: L. V. Taylor, American Can Co., Maywood, Ill.

DENATURANTS IN DISTILLED SPIRITS:

Associate referee: G. F. Beyer, Bureau of Internal Revenue, Washington, D. C.

WHISKEY AND RUM:

Associate referee: Peter Valaer, Bureau of Internal Revenue, Washington, D. C.

DETECTION OF ADULTERATION IN DISTILLED SPIRITS:

Associate referee: S. T. Schicktanz, Bureau of Internal Revenue, Washington, D. C.

WOOD ALCOHOL IN DISTILLED SPIRITS:

Associate referee: G. F. Beyer.

CORDIALS AND LIQUEURS:

Associate referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

FOOD PRESERVATIVES—SACCHARIN:

General referee: W. F. Reindollar, Bureau of Chemistry, Baltimore, Md.

BENZOATE OF SODA:

Associate referee: A. E. Mix, Food and Drug Adm., Washington, D. C.

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WILEY MEMORIAL LECTURE. No. IX

A NEW LAW BRINGS NEW PROBLEMS

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It seems eminently fitting that the final paper in the Wiley Memorial series before this Association should deal with regulatory matters. Dr. Harvey W. Wiley was the motive power behind the passage of the Food and Drugs Act of 1906, and for a third of a century the activities of this Association have been indelibly stamped with a regulatory cast because of that legislation. Now, after a legislative struggle comparable in some respects with the historical battle waged by Dr. Wiley, a new Federal Food, Drug, and Cosmetic Act replaces the act of 1906. The annals of this Association should contain more than a passing record of this new legislation.

In November 1932, I presented to this Association a paper entitled "The Never Ending Problems of the Regulatory Chemist." For today's discussion I have quite naturally selected as a title "A New Law Brings New Problems." What I wish to present to you is, first, a summary of the new law in comparison with the former statute; second, a brief report of what has been accomplished during the short period the law has been partly in effect; and finally, the challenge and opportunity the new law offers this Association, which I am confident it will meet in the same fashion that it met the challenge of the earlier law.

The Federal Food, Drug, and Cosmetic Act received presidential approval on June 25, 1938. An amendment postponing certain provisions was approved June 23, 1939. It has been more or less customary to date the history of this new legislation from the introduction in the 73d Congress on June 6, 1933, by Senator Royal S. Copeland, of the bill known as S. 1944. That bill, it is true, was the forerunner of the present law. It underwent many changes; in some respects was weakened and in many respects improved; but fundamentally it contains most of the essentials long recognized as necessary for reasonably adequate public protection.

To keep the record straight let me point out that the battle for more effective food, drug, and cosmetic legislation did not begin with the introduction of the Copeland bill. Officials connected with enforcement of the Food and Drugs Act, and particularly the present Chief of the Food and Drug Administration, have consistently and repeatedly advocated amendatory legislation. The annual report of the Food and Drug Administration for 1931 reviewed the long-recognized deficiencies of the act of 1906 and made definite and specific recommendations for essential amendments. Some of these were in fact submitted to the Congress in the form of bills. Every one of these recommendations is now incorporated in the Federal Food, Drug, and Cosmetic Act.

While there are pronounced differences between the act of 1938 and the older statute, the former preserves all of the valuable features of the original measure. The philosophy of Congress was quite definitely to protect the public health at every stage in the traffic in foods, drugs, and cosmetics; to guarantee comprehensive information to the consuming public as to the character and composition of foods and drugs; to protect consumers against deception; to facilitate enforcement operations by removing some of the legislative handicaps of the earlier law; and to impose penalties that will constitute genuine restrictions against violations of the statute.

The law as originally passed was to have become effective in its entirety on June 25, 1939, although three sections—those relating to new drugs, to dangerous drugs, and to poisonous cosmetics—became effective on the date of its approval. The amendment of June 23, 1939, postponed the effective date of many of the new labeling requirements and certain other provisions until January 1, 1940, with limited authority to the Secretary of Agriculture in special cases for further postponement to July 1, 1940.

Let me summarize the requirements of the new law as compared with those of the older statute. Not only is the scope of the act extended to cover cosmetics and curative and diagnostic devices, but the definition of the term "drug" is enlarged. In addition to therapeutic agents, it now includes diagnostic materials and those substances commonly regarded as drugs but which are intended to alter or affect some normal function of the body. These were not subject to control under the limited definition of "drug" in the old act. The new law materially broadens the definitions of adulteration and misbranding. It removes the burden of proving fraud in actions against drug preparations bearing therapeutic claims.

The food section of the statute contains no more important provision than the authority for the establishment of reasonable definitions and standards of identity and reasonable standards of quality and fill of container to promote honesty and fair dealing in the interest of consumers. The authority so granted is nothing less than the substantial cornerstone of effective enforcement of the food sections of the act. Lack of such authority in the act of 1906 was unmistakably the most serious handicap to effective protection of the food supply against debasement.

Another valuable provision specifically relating to food adulteration is the prohibition of traffic in food which may per se be injurious to health. The act of 1906 merely prohibited traffic in food injurious because of the presence of an added poisonous or deleterious substance. Invaluable is the authority conferred for the establishment of tolerances where added poisons are the unavoidable result of good manufacturing practices. Other public health provisions applicable to foods, not included in the former law, provide for action in the case of a product prepared, packed, or held under insanitary conditions whereby it may have become contaminated

with filth or may have been rendered injurious to health, and against a food if its container is composed, in whole or in part, of any poisonous or deleterious substance which may render the contents injurious to health. The food section contains a special provision to guarantee the purity of confectionery and a provision, repeated also in the drug and cosmetic sections, requiring that only such coal-tar colors may be used as have been certified by the Secretary of Agriculture as safe and suitable for use.

The misbranding section under food retains substantially the requirements imposed under the old statute but eliminates the troublesome distinctive-name joker; calls for a positive declaration of the name and address of the responsible manufacturer, packer, or distributor; requires imitations to be specifically so labeled; and requires a food purporting to be or represented as one for which a definition or standard of identity has been prescribed to comply with this standard and to conform likewise with the quality standard, if one has been established, unless suitably labeled to show that it falls below such quality standard. Of outstanding importance is the requirement that fabricated foods for which no standard of identity has been set up shall bear the common or usual names of their ingredients. Provisions for informative labeling of special dietary articles and for adequate declaration of artificial flavoring, coloring, and chemical preservatives, and a ban against slack-filled and deceptive containers are included.

The sections applicable to drugs contain provisions comparable to those in the food section relating to filth and decomposition, injurious and deceptive containers, and certified coal-tar colors. They make adequate provision for compliance of drug products with existing United States Pharmacopoeia and National Formulary standards, and provide for types of informative labeling greatly in excess of those provided by the law of 1906. Habit-forming drugs specified in the law must be labeled with warning statements that they are habit-forming; certain potent ingredients, also specified, must be quantitatively declared; all potent ingredients in non-standardized drugs must be qualitatively stated, and adequate directions and warnings against misuse must be incorporated upon the labels. Any drug or device that is dangerous to health when used in the dosage, or with the frequency or duration prescribed, recommended, or suggested in its labeling is outlawed.

Perhaps no feature of the new law has received more attention from the drug industry than that prohibiting the introduction of new drugs into interstate commerce unless an application has been filed with the Secretary establishing that the article is safe for use. This is the section enacted by Congress as a means of preventing future tragedies like the elixir sulfanilamide disaster. Almost with the passage of the act drug manufacturers began to file applications covering new drugs. The great majority of these applications covered merely new combinations of known drugs,

innocuous from a public health standpoint at least insofar as direct damage to health may be alleged. In many instances, however, it is highly questionable whether they possess the therapeutic properties claimed for them; consequently, even though the new drug compound may not be actionable under the new drug section of the act, proceedings against many such products because of unfounded therapeutic claims may occur after the law becomes fully effective. Applications covering new drug products of this description can be and are promptly reviewed from the standpoint of possible injury to health and are accepted as provided by the new drug section without undue delay. There are, however, other medicinal preparations of a distinctly new, possibly highly valuable kind, developed by research laboratories on which far more deliberate and detailed consideration is necessary before it can be determined that an application for authority to introduce the product into interstate commerce may be accepted by the Secretary.

The Food and Drug Administration has a keen sense of the solemn responsibility imposed upon it by the new drug section. It realizes on the one hand that any steps that may result in withholding a valuable drug from an ailing public may result in untold suffering, while on the other hand any undue haste in releasing a drug that may be potentially dangerous may likewise prove disastrous. As an indication of the thoroughness with which new drug applications of this type must be considered it may be stated that before favorable action could be taken on a recent application covering an important new drug, medical officers of the Food and Drug Administration made comprehensive reviews of the records of more than 2,000 cases reported by approximately 100 physicians.

The provisions of the new act relating to cosmetics are, of course, entirely new. Cosmetics have not heretofore been subject to Federal control. The provisions are quite similar in their scope to the requirements of the old act applicable to foods and drugs and are comparatively simple. Emphasis is placed upon the exclusion of dangerous cosmetics or those that are filthy or otherwise unfit for use. Only certified coal-tar colors may be used except in the case of hair dyes, which must bear specified warning labels and directions for appropriate sensitivity tests. The branding section provides for such fundamental information as the quantity of contents and the name and address of the manufacturer, packer, or distributor, together with a blanket prohibition on false and misleading statements of every description. As in the case of foods and drugs, containers so made, formed, or filled as to be misleading are outlawed.

One portion of the new law, section 201 (n) is entirely novel and will have a most profound influence upon the interpretation of misbranding. Under the old law a food or drug was deemed to be misbranded if its labeling was false or misleading in any particular. Under the new law a product may be misbranded because of what the label fails to say as well as be-

cause of the inaccuracy of what it does say. Under the new law the enforcing officer must take into consideration the extent to which there is a failure to reveal material facts as well as the extent to which false and misleading statements are incorporated in the labeling.

Enforcement procedure is materially amplified as compared with the act of 1906. The new law provides for injunction proceedings. It grants extraordinary emergency powers to be used in case of necessity to prevent interstate traffic in contaminated and dangerous food. It authorizes factory inspections and the acquirement of records of interstate commerce, and imposes penalties commensurate with the character of the offense instead of the inadequate penalties authorized under the former statute. Time permits only this hasty and incomplete analysis of some of the features of the new law.

Let me now give you a brief report of what the Food and Drug Administration has accomplished under the Food, Drug, and Cosmetic Act since June 25, 1938, the date of its approval. The new law made instantly effective those provisions intended to protect the public health against dangerous drugs, devices, and cosmetics. It granted authority to begin immediate formulation of necessary regulations, including definitions and standards for foods, continuing in force meanwhile the provisions of the act of 1906 until the new law became fully effective.

Legal actions instituted under the new law during the first year following its passage were directed against dangerous drugs, devices, and cosmetics. Within three weeks after approval of the act interstate shipments of sight-destroying eyelash dyes containing paraphenylenediamine or related compounds were located, sampled, and analyzed, and seizures were instituted. The objective was to drive such products off the market. Sixty-five seizures were made. Criminal prosecutions were directed against responsible shippers, and for the most part these have been terminated with pleas and the imposition of fines and in some cases suspended prison sentences. Seizures of eyelash dyes containing ammoniacal silver salts and pyrogallol were also made in the conviction that such products are dangerous to the eyes. The manufacturer of one of these articles contested the action and a three weeks' trial resulted in a jury disagreement. This case will be retried. Other dangerous cosmetics that have led to legal action since the passage of the act are skin-bleaching creams containing mercury compounds, lipsticks containing cadmium and selenium, and mole removers consisting essentially of acetic and nitric acids.

Among the drug products held to be adulterated because dangerous to health when used in the dosage, or with the frequency or duration prescribed, recommended, or suggested in the labeling, were products of the pain- and headache-reliever type containing such potent drugs as cinchophen, aminopyrine, barbituric acid derivatives, and combinations of bromides and acetanilids, as well as obesity treatments containing desic-

cated thyroid. While most of the cases so instituted have already terminated by default or consent decrees, several court contests are in prospect.

I shall not take time to describe a variety of actions directed against dangerous devices subject to the act, nor do more than mention numerous proceedings instituted in the case of deceptive and slack-filled containers after June 25, 1939, when the section of the law prohibiting such containers went into effect. From the standpoint of fraud and deception I know of nothing more striking than some of the malpractices involved in deceptive containers. It was high time that legal control over such articles was undertaken. As a matter of statistics let me say that between June 25, 1938, and October 27, 1939 (last Friday, that is), a total of 674 legal actions were instituted under the new law covering 150 shipments of drugs, 57 of devices, 316 of foods, and 151 of cosmetics.

Drug manufacturers began to file applications under the so-called new-drug section of the act immediately after its passage. Between June 25, 1938, and October 27, 1939, 1,796 such applications had been received, 1,128 had been made effective, 475 were held pending a revision of the applications by the applicants, 65 were in course of intensive review in the Administration, 75 had been withdrawn by the applicants (usually upon recognition that the applications would be refused for lack of sufficient evidence of safety), and 1 had been definitely denied. At the time these figures were compiled only 52 of the 1,796 applications received had not yet received consideration in the Administration.

But the institution of court actions and the survey of new-drug applications represent only two of many obligations imposed upon the Administration by the new law. Many sections of the act call for the formulation of special regulations. In collaboration with the Solicitor's office of the Department, many of these regulations have been formulated and after public hearings have been promulgated by the Secretary of Agriculture. These include general administrative regulations for the enforcement of the statute, regulations relating to imported products, to coal-tar color certification, and to canned seafoods packed under inspection.

A list of 132 coal-tar colors and their lakes, found after careful study to be harmless and suitable for use in foods, drugs, and/or cosmetics, has already been set up, and 31 additional colors are now under consideration as possible additions to this list. Between May 11, 1939, when color certification began, and October 27, 1939, more than 300,000 pounds of straight or primary dyes and 279,000 pounds of color mixtures were certified after chemical examination as suitable for coloring foods, drugs, and/or cosmetics.

The formulation of regulations prescribing definitions and standards for food products has called for much detailed work. To facilitate standard-making operations, a Standards Committee was set up in the Food and Drug Administration, composed of four experienced State food law

enforcement officers and two representatives of the Food and Drug Administration. Let me pay tribute to the wholehearted and generous cooperation that State officials have given to the Food and Drug Administration in this and many other enforcement activities. The Standards Committee, in cooperation with the administrative officers of the Administration, determines what foods shall be standardized, evaluates available data in support of a definition and standard, outlines to the field forces of the Administration additional data to be acquired, obtains the views of consumers and the trade, and eventually formulates tentative specifications for definitions and standards. These specifications are considered by the Administration and with the aid of the Solicitor's office are placed in proper form for announcement of public hearings before a representative of the Secretary. The Secretary's representative, after each hearing, makes a suggested finding of fact and formulates a suggested regulation. These are published for comment and criticism by interested parties. After ample time for such comment and criticism has been allowed, the entire record goes before the Secretary for the issuance of the order promulgating his findings of fact and a regulation prescribing a formal definition and standard. Proposed definitions and standards of identity, or of identity and quality, or of identity, quality, and fill of container, have already been announced for a wide variety of food products, and final definitions and standards have been promulgated after hearings in the case of numerous commodities.

Let me now turn to the enlarging problems of this Association growing out of the enactment of the new statute. The Association of Official Agricultural Chemists has little reason for existence except to supply the tools for the enforcement official and the agricultural chemist, whether he be interested in foods or drugs, in feeds or fertilizers, in insecticides, or in naval stores. The Association is not concerned with the collection of data for the sake of adding to the sum total of human knowledge. It is not interested in abstract scientific research. It is concerned with these things solely as they may be utilized in the formulation of methods available for law enforcement operations and agricultural chemical analysis. The very name of the Association—The Association of Official Agricultural Chemists—is in a sense a misnomer unless we admit that the chemist has usurped the field of the bacteriologist, physicist, microanalyst, and many other branches of science. Note, for example, that the official book of methods contains such non-chemical procedures as the grading of rosin and the examination of tomato products for molds, yeasts, and bacteria. The inclusion of such procedures in the book of methods is merely an acknowledgment of the fact that the Association is alive to its opportunities and aware of its obligations. Any technic for examining and evaluating a commodity subject to regulatory control is proper for study by this Association. The Association is now confronted, in the enactment of this

new statute, by a challenge and an opportunity. No longer is the jurisdiction of the Food and Drugs Act confined to foods and drugs. Cosmetics; curative and diagnostic devices; chemicals intended for diagnostic procedure, for affecting the function or structure of the body other than in the treatment of disease; and food, drug, and cosmetic containers now come within the scope of the statute.

In the paper, "The Never Ending Problems of the Regulatory Chemist," already referred to, I tried to depict the eternal variety of the technical problems confronting the regulatory chemist and the unfailing urge for the exercise of ingenuity in devising new methods of attack. Let me catalogue only a few of the problems awaiting solution. We need more and better methods for the estimation of filth and decomposition in foods, drugs, and cosmetics, for the estimation of vitamins, for the identification of coal-tar dyes, for the estimation of heavy metals and toxic ingredients of many kinds, for the chemical and bio-assay evaluation of drugs, for the identification of many common ingredients of food mixtures, and for the analysis of cosmetics. We should have more and better methods of examining glandular preparations, of evaluating devices, and of estimating the degree of deceptiveness in containers of many types. The contribution this Association can make in this field is unlimited. The value in court actions of methods bearing the stamp of its official approval is self-evident.

In pointing out these opportunities for valuable work I cannot refrain from repeating some of the suggestions made in my previous paper. I quoted the presidential address delivered by my dear friend, Mr. R. E. Doolittle, at the 40th annual convention of the A.O.A.C. in 1924, in which he discussed "The Needs of Our Association." "The most important problem," said Mr. Doolittle, "that confronts the Association as I study the situation, is a proper coordination of its activities. Little difficulty is experienced in securing competent chemists to take charge of its investigative and collaborative studies but a Referee or Associate Referee, once selected, is left almost entirely to his own resources." Mr. Doolittle then went on to outline his views regarding the procedure of systematization that would insure more coordinated and profitable work on the part of the referees. It has been my own observation that notwithstanding the sound common sense and logic exhibited in the plan proposed by Mr. Doolittle, it has been largely neglected in the 15 years since it was presented. No one is particularly to blame for this situation. An organization conducted on a voluntary basis by workers who ordinarily can contribute to the collaborative work only at the sacrifice of private time cannot be expected to conform with military precision to a systematic plan of operation no matter how logical it is. The subcommittees dealing with referees' reports and recommendations are ordinarily obliged to assemble hastily and rush through an exhausting amount of detail work in the review of recommen-

dations and methods that should be undertaken with far more deliberation. It is surprising that the work is so adequately performed. To my way of thinking the time has come when this Association should begin to think seriously about the part-time employment out of its own funds of one or two well-trained chemists who have an understanding of the needs of the regulatory operator, and the ability to direct the referees by inspiring their confidence and inducing them to seek their advice and direction. What I have in mind is not to supplant but to give some measure of assistance to those men who have so loyally sacrified their time and strength without compensation and too often without thanks or appreciation, in directing the work of the referees and in carrying on other arduous duties connected with this Association's projects. If I am correct in my conviction that the scope of the Association's activities has been greatly enlarged by the passage of this new statute I can conceive of no way that the challenge can be adequately met without some definite action of the kind I have suggested on the part of this Association.

In my earlier paper I pointed with pride to the fact that the then current book of methods contained 564 pages as compared with 230 in Bulletin 107, the immediate predecessor of the official book of methods. The present book of methods, the Fourth Edition, appearing in 1935, contains 710 pages. I shall be surprised and disappointed if 10 years hence a further material increase, due to the inclusion of substantial and valuable new methods, has not occurred, for the enactment of June 25, 1938, has opened up a vista of opportunity that cannot, and I am sure will not, be neglected by this organization.

PRESIDENT'S ADDRESS*

FOOD STANDARDS

By W. S. Frisbie
(U. S. Food and Drug Administration, Washington, D. C.)

Most presidential addresses, by common consent, are expected to sound a keynote, but for this meeting the keynote was struck over 50 years ago—in 1884 to be exact—when that small group of chemists met in Philadelphia and organized the Association of Official Agricultural Chemists, for in the constitution then adopted they declared as their main objective accuracy and uniformity in methods of chemical analysis. True it was that in those first years the members discussed solely methods of fertilizer analysis, but we know the constitution was soon amended to include soils, cattle feeds, dairy products, and other materials connected with agricultural industry.

Therefore, to justify these remarks there should be some text—perhaps a pretext—other than to register a due sense of appreciation to this Association for my election to this office; and this I do in all sincerity. I shall therefore record the participation of our Association in the formulation of food standards and endeavor to show the increasing importance of those methods of analysis that have to do with these standards.

Between the year 1886, when the first constitutional amendments were adopted, and the year 1890 it appears that some work was done on dairy products as well as attention given to methods of analysis for sugars and for wines. The question of food products in general and methods for detecting their adulteration seems not to have been seriously considered until 1895 when the president of the Association that year observed that food for soils and for cattle had received attention but food for human beings had not. He called attention to the reckless manner in which all sorts of substances were added to foods either to prevent fermentation or to conceal bad quality. He stated, "For, while our reports abound in researches on food for live stock, the only work on the food for man is represented in the reports on dairy products and sugar, and as a rule comparatively few of us contribute to either of these branches."

Apparently the Association agreed with its president for we find that a resolution was adopted that year and a committee appointed to consider the position the Association would take with reference to food adulteration and legislation relating thereto.

In 1896 the president stated, "At the last annual meeting of this Association the president in his opening address called attention to the importance of the study and investigation of foods for human consumption, and pursuant to a resolution adopted at that time a committee was appointed

* Presented Monday afternoon, October 30, 1939.

to consider what attitude it is desirable for this body to assume with reference to food adulterations and legislation relating thereto, and a report from this committee will no doubt be presented in due order."

A definite report was made to the Association the next year (1897) when Dr. William Frear, who was then president, called attention to the fact that records of food control proved that 5 to 15 per cent of the entire food supply was adulterated and that 10 per cent of such adulteration was injurious to health. He recommended that steps be taken "to secure the establishment of standards of composition for pure food substances just as the druggists have done for drugs." He continued: "The United States Pharmacopoeia is the accepted authority as to standards for the purity of drugs, and incidentally for a few food substances. Most state and municipal laws relating to the control of drug adulteration distinctly accept the pharmacopoeial standards of purity. A similar work must be done for foods. In the absence of such an accepted set of standards each food chemist is compelled to formulate his own. The result is that the standards used by different chemists are based upon insufficient data and are formulated according to different canons, with the consequence that legal actions are instituted in good faith by control officers only to be lost by the failure of the several experts to agree upon the essential bases of comparison".

At this meeting, on the 28th of October, 1897, the Association appointed a committee on food standards, the first committee of its kind in the United States. The personnel: Harvey W. Wiley, chairman; H. A. Weber of Ohio; M. A. Scovell of Kentucky; E. H. Jenkins of Connecticut; and William Frear of Pennsylvania.

In 1900, while the personnel of the committee remained unchanged, Dr. Frear succeeded Dr. Wiley as chairman and reported that definitions and standards had been adopted tentatively for the following classes of products: Meats; lard; milk and milk products, including condensed milk and ice cream; jams and jellies; sugars; sirups; and four flavoring extracts—cinnamon, peppermint, lemon, and orange.

In 1902 the committee reported that it had ready for submission tentative definitions and standards for the previous list, which had been supplemented by schedules for fruit juices, tea, coffee, and cocoa.

In 1903 the committee reported that a number of definitions and standards had been revised and prepared for submission to the members of this Association, to dairy and food commissioners, and to the trade for comment and criticism; and that it expected to have completed by the end of that year its work on final definitions and standards for those products first given consideration.

The work of this committee was materially aided by the Act of Congress in 1902 authorizing the Secretary of Agriculture, in collaboration with the Committee on Food Standards of the Association of Official Agri-

cultural Chemists, to fix standards of purity for foods and to determine their adulteration. Funds for this work were included in the appropriation for the Department of Agriculture. From this time until this clause in the appropriation act was discontinued, shortly before the Act of 1906 was passed, the expenses of the committee were paid by the Department of Agriculture.

After the Food and Drugs Act was signed, June 30, 1906, the Secretary of Agriculture appointed a committee that was known as the U. S. Commission on Purity of Foods; it combined the A.O.A.C. Committee on Food Standards with one that had been appointed by and for the Association of State and National Food and Dairy Departments. It should be noted here that our Association continued its own Committee on Food Standards, still with the personnel unchanged. The records show that this Commission or joint committee held one meeting at which time consideration was given to meat extracts, fruit juices, fruit sirups, and malt and spiritous liquors. In view of the last item it seems appropriate that this meeting took place in Louisville in December, 1906. That this committee was nevertheless serious-minded is attested by the following: "Resolved: That if the Commission visit any distilleries outside of Louisville, these visits be made at the end of the session."

Following this meeting, the A.O.A.C. committee in 1907 published a report recommending the adoption of standards for meat extracts, fruit juices, spiritous liquors, preservatives, and colors.

The Joint Committee of the A.O.A.C. and Association of State and National Food and Dairy Departments met at Jamestown, Va., in 1907. The result of this meeting was the adoption of a revision of the schedules that had been recommended the previous year.

The committee of this Association again met with the committee from the Association of State and National Food and Dairy Departments in 1908 at Mackinac, and, as a result, those schedules that had received their joint approval were published in separate bulletins, both by the A.O.A.C. and by the state officials, but not by the Department of Agriculture.

We find no record of a meeting in 1910 and no report of this Association's committee. A meeting of the Joint Committee was called for August, 1911, but no records are available as to whether or not such a meeting took place and again no report is found of the Committee on Food to this Association. However, according to the records of the A.O.A.C. the original committee continued to function until 1913, when the Secretary of Agriculture in response to requests from state food officials appointed a Joint Committee on Definitions and Standards to consist of nine members, three from the Department of Agriculture, three from the Association of Dairy, Food and Drug Officials, and three from the Association of Official Agricultural Chemists. The A.O.A.C. members of the committee appointed November, 1913, were William Frear, Julius

Hortvet, and John P. Street. This committee met at least once annually, usually more often, uninterruptedly until 1933. That committee held its last meeting in 1937. When this joint committee was organized, the Committee on Food Standards of the A.O.A.C. was dissolved after a record of 16 years of continuous service to this organization, and during all those years with only one change in personnel, and for 13 years under the chairmanship of Dr. Frear. This committee formulated 212 definitions and standards, which were published by the Department of Agriculture in Office of the Secretary Circular 19, June 26, 1906. When we realize that at the close of 1937 the total number of definitions and standards was approximately 300, we must recognize the industry and energy that characterized the members of this Association's committee.

The newly formed Joint Committee did not lose sight of the extent and value of the contributions to food standards that had been made by the A.O.A.C. committee. This is evidenced by the fact that for the 10 years following 1913, before any schedule or any definition or standard for an article of food was submitted to the Secretary of Agriculture for approval, it must have received the endorsement of the A.O.A.C. in annual session. Since this procedure was found to delay in some instances the adoption of much needed schedules, this Association voted to confer upon its representatives on this joint committee the right to speak for and to act for the Association in all matters pertaining to food standards.

When the Federal Food, Drug, and Cosmetic Act was passed in 1938, logically the Joint Committee appointed under the Act of 1906 no longer had any official status. In August, 1938, a new committee was appointed by the Secretary of Agriculture to function within and for the Food and Drug Administration in the formulation of standards for foods, which under this present Act are now to have the force and effect of statute law. We all know, even though the size of the committee was reduced, that the A.O.A.C. representatives still consitute one-third of that body.

In this connection it is interesting to revert to a prophecy made by a president of this Association just 50 years ago. Professor John A. Myers, in 1889, in his presidential address, said: "It (the A.O.A.C.) is aiming to lay a foundation so solid that every court in this land must respect its conclusions, and every analytical chemist, whether he lives in this country or elsewhere, must be forced either to practice or admit the advantages and correctness of our system of analyses."

While the methods of analysis of this Association had received judicial notice, they still could not be regarded as compelling recognition by the Courts. In the procedure now established for promulgation of food standards we find incorporated in each definition and standard for which numerical limits are set, or for which methods of analysis are essential in determining validity, the applicable methods of analysis of the A.O.A.C. In those schedules, which already have been prescribed by regu-

lations of the Secretary, i.e., those for tomato products and those for egg products, there have been included as an essential part of the definition and standard the methods of analysis of this Association.

As the number of foods for which standards must be fixed continues to multiply and our methods of analysis are incorporated, this service alone will call for a continuance of that exacting work through which this Association has achieved a unique reputation, both in this and in other countries of the world.

Unless set aside by appeal and subsequent judicial order these methods, which are a part of the definitions and standards for foods, will become the only legal procedures for the determination of those constants for which they are prescribed. Thus in a measure has the prophecy of Professor Myers been fulfilled, and in this consummation our Association ought to take justifiable pride, not only in the recognition of its methods in legal food standards but in the contribution it has made toward the establishment of those standards for regulatory purposes.

While we regard with due reverence those members of this Association who have passed on and do appreciate in full their dedication to this important work, we must not forget the living, who have faithfully continued and still continue to follow the traditions of these pioneers. While Dr. E. M. Bailey is not a member of the present committee, his 15 years of diligent service on the former committee is known to you all. You need hardly be reminded of your representatives on the present committee —Guy G. Frary of South Dakota and Charles D. Howard of New Hampshire, who incidentally has now given 21 years of service in this capacity. By the same token this organization is a living one, but if it is to be kept alive it must have new blood; this, of course, must come from those chemists that represent the younger members of the Association. The opportunity for active participation in the varied kinds of work afforded by this Association should constitute a challenge, particularly to those chemists that have elected to enter the fields of public service dependent upon scientific regulation and control.

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 then follow in the usual order.

THIRD DAY

WEDNESDAY—AFTERNOON SESSION

REPORT OF EDITORIAL BOARD

By W. W. SKINNER, *Chairman*

The work of the Editorial Board has proceeded along the lines followed in previous years, so my report is merely one of progress. The 5,000 copies of *Methods of Analysis*, 4th edition, was exhausted six months before the end of the five year revision period, which is something that has never occurred before. As you know, 1940 is the year when the next revision should be made—the 5th, or 1940 edition. At the annual meeting of the Executive Committee it was decided that in view of the anticipated increased demand for the books there should be published 7,000 copies of the next issue. Mr. Lepper will report on *The Journal*, and Dr. Bailey will report specifically on the revision work done on *Methods of Analysis*.

The exhaustion of the 4th edition has made it possible to sell some of the 300 copies of the 3rd edition, which the Secretary had been authorized to distribute to libraries, because it was thought there would be no further use for them. Since July we have sold approximately 180 copies of these books.

The material for the Wiley books that was held by the printer was purchased by the Association because the printer was going out of business. It was brought to Washington, collected, and bound in volume form, and copies are now available. There has been a moderate demand for these books and we expect that the amount expended, about $500.00, will be realized from the sale.

Approved.

REPORT OF THE EDITORIAL COMMITTEE OF *THE JOURNAL*

By HENRY A. LEPPER, *Editor*

The Journal was originally created as the medium of publication for the proceedings of our Association. Gradually as it advanced in years it opened its pages more and more to the dissemination of reports on other

original investigations in agricultural chemistry and related subjects through its section on contributed papers. How well it has fulfilled its mission is evidenced by the 800 pages in the volume for this year, the largest single volume we have ever issued. This has been done, as the report of the Secretary-Treasurer will show, on a self-supporting basis. The accomplishment is one of gratification, but also some disappointment. It is gratifying because this growth is a result of an enlarged contribution from referee reports and shows a stimulated interest in the work of the Association. It is disappointing because the section on contributed papers has not kept pace this year with the general enlargement, being about 30 pages below the number devoted to the section in the past few years. The ever expanding interests of the Association are reflected in the appearance this year of contributed papers on cosmetics. The financial status of *The Journal* permits an expansion of this section, and members should avail themselves further of the opportunity to publish the results of their investigations in the pages of their *Journal.*

Approved.

REPORT OF COMMITTEE ON REVISION OF METHODS

By E. M. BAILEY, *Chairman*

The Committee submits the following report on the progress of work on the 1940 edition of our *Methods of Analysis.*

All changes in methods adopted since the 1935 edition have been incorporated in the present text and the chapters thus revised sent to the various referees for their review. A list of general comments for the guidance of referees was sent with each chapter, and in most cases a list of special comments or suggestions calling attention to specific matters of text upon which the committee desired advice or information. Practically all of these chapters have now been returned with the information sought, and with additional comments helpful to the committee.

With this material on hand we are brought up to the date of our present meeting. The next step is to review the proceedings of our present session and incorporate the further additions and changes that have been adopted.

In recent editions it has been a recurring problem with revision committees to keep the volume of our text within such limits that its purchase price may be kept at a reasonable figure. When we consider the scope of our work and the number of new methods adopted each year, some increase in the size of the book at each 5-year interval is inevitable, especially if the text is to meet the increasing needs of those whom it is intended to serve. But reasonable space-saving is in order. Devices to this end have been adopted in the past and more will be adopted for the

revision in hand. Reduction in size of cuts wherever possible, utilization of blank space where text may be inserted, reduction in volume of preparatory matter, and other changes in the physical set-up will effect considerable economy. The chapters have already been edited on the basis of the deletion of the articles "a," "an," and "the" wherever accuracy and clarity will not be sacrificed. In general there has been no adverse comment on this device; some are opposed, but on the other hand, there is, from other members, the urge to go even further in this direction.

The Committee's attention has been called to the rather wide misuse of the word "strength" when concentration is the intended meaning; and to the less frequent misuse of the word "test" when applied to a quantitative rather than a qualitative procedure. It has been proposed to make Appendix I, Preparation of Standard Solutions, a separate chapter, and to substitute the abbreviation "ml" in all cases for "cc." All of these proposals will be given due consideration.

The Committee has sought to have all matters involving changes in methods recommended by the respective referees in order that they may go through the regular channels of consideration and be acted upon in due course.

Those of you who have served on revision committees in the past will appreciate that there is still much work to be done. We can venture no prediction as to when the new book will be in your hands, but arrangements have been agreed upon within the Committee to clear up what remains to be done as rapidly as possible. The Committee is indebted to the fine cooperation of the various referees for their help in all of this work.

Approved.

No report was given by the Committee on Quartz Plate Standardization and Normal Weight.

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

Official, First Action

CALCIUM NITRATE

Calcium nitrate (nitrate of lime) is a commercial product consisting chiefly of calcium nitrate, and it shall contain not less than fifteen per cent (15%) of nitrogen.

AMMONIATED SUPERPHOSPHATE

Ammoniated superphosphate is the product obtained when superphosphate is treated with ammonia or with a solution containing free ammonia and other forms of nitrogen dissolved therein.

SUPERPHOSPHATE

Superphosphate is a commercial phosphate, the phosphoric acid (P_2O_5) content of which is due chiefly to monocalcium phosphate. (The grade that shows the available phosphoric acid should always be used as a prefix to the name. *Example:* 16 per cent superphosphate.)

First Reading as Tentative

NITRATE OF SODA AND POTASH

Nitrate of soda and potash is a commercial product containing nitrates of sodium and potassium. It shall contain not less than fourteen per cent (14%) of nitrogen (N) and fourteen per cent (14%) of potash (K_2O).

GUARANTEEING IN TERMS OF ELEMENTS

With the exception of potash (K_2O) and phosphoric acid (P_2O_5) the other ingredients of fertilizers, such as calcium, magnesium, sulfur, manganese, copper, zinc, and boron, shall be guaranteed in terms of the elements.

Proposed Definitions (Resolution)

Resolved, that in the brand name of fertilizer materials the grade that shows the available primary fertilizer component should always be used as a prefix to the name, for example: 18 per cent superphosphate; 20 per cent kainit; 60 per cent muriate of potash.

MAGNESIUM OXIDE

L. S. WALKER, *Chairman*
H. D. HASKINS
W. C. JONES
G. S. FRAPS
L. E. BOPST
W. H. MACINTIRE

Approved.

REPORT OF COMMITTEE ON RECOMMENDATIONS OF REFEREES

By HENRY A. LEPPER, *Chairman*

In anticipation of scheduled revision of the methods of analysis of the Association for 1940 your Committee last year pointed out in its report the responsibilities the passage of the Federal Food, Drug, and Cosmetic Act has placed on us to improve our methods in fundamentals directed toward greater accuracy, precision, or economy of time. The often expressed plea for uniformity of procedures to eliminate repetition in directions throughout the book was repeated. The report, in the form of a reprint, was sent to all referees and associate referees with a letter in May, 1939, discussing further the task of revision and suggesting approaches for improvement in directions and extended application of procedures. Much of this advancement has been accomplished this year, but at the same time it is to be regretted that the progress on unification of procedures has been very limited. The principles established by the Association for the adoption of methods as official only after evidence of

satisfactory collaborative application precludes changes in the directions after recommendation for adoption. The details must be those of the directions tried by the collaborators. Unification involving changes cannot therefore be accomplished by the Committee on Revision of Methods or by this Committee. The responsibility is that of the referees. Closer coordination and cooperation between referees preparatory to studies for the determination of the same constituent in different products would often lead to an agreement on fundamentals with only differences in adaptation before methods are outlined and proposals made to collaborators. Again the application of established procedures already available will operate to limit repetition of directions for the determination of the same constituent. Your Committee again urges all referees and associate referees to give particular attention to the possibilities in the direction of unification. It is an important consideration in the ever expanding scope of our work.

During the last few years we have made an attempt at unification on one constituent, moisture, through the work of a committee on moisture. This approach to the problem has not proved successful and members of the committee have recommended that its work be discontinued and that individual referees continue the subject as applied to their respective products. It is therefore recommended that the committee on moisture be discontinued.

Practically every year sees an expansion in the activities of the Association. This year is no exception. We have recommended a program of study for cosmetics and colors in drugs and cosmetics calling for the appointment of two general referees and twelve associate referees. The work on drugs has expanded so that recommendations will be made to provide four general referees on drugs, in place of the one now officiating. This year sees the inauguration of bacteriological procedures for sugars, dried milk, and canned vegetables in our adopted methods. A reassignment of subjects among the subcommittees is contemplated.

In connection with the revision of methods of analysis it is recommended that the changes that have been or will be made be referred to the referees for clarification of procedures and that those that are editorial in character be approved by the Revisions Committee and concurred in by this Committee without final presentation in detail.

The Referee on Fertilizers has recommended that the Association recommend the testing of flasks, burets, pipets, and weights used for analyses for fertilizers, which have not been tested by the Bureau of Standards, and consider the adoption of methods for testing them or appoint an associate referee to recommend methods for testing unstandardized flasks, burets, pipets, and weights, and for checking the accuracy of weights in use.

This recommendation has been regarded by your Committee as one in-

volving all methods of the Association, and it was presented for consideration by the total membership of this Committee rather than for action by Subcommittee A, to which recommendations of the Referee on Fertilizers are customarily referred. In the opinion of the Committee this question involves a question of policy in extending the scope of our methods. It does not appear to be a question for consideration of an associate referee but rather one for study by a special committee. We recommend the appointment of such committee for report to the Executive Committee before the next meeting. (See p. 97.)

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES*

By G. E. GRATTAN (Department of Agriculture, Ottawa, Canada), *Chairman;* H. A. HALVORSON, and E. L. GRIFFIN

STANDARD SOLUTIONS

It is recommended—

(1) That, as amended this year, the method for standardization of acid solutions with borax recommended by the associate referee (*This Journal,* **22,** 102) be adopted as official (final action).

(2) That the method submitted by the associate referee for standardization of acid solutions with sodium carbonate (*This Journal,* **22,** 103) be adopted as official (final action).

(3) That collaborative work be done on the method submitted by the associate referee last year for the standardization of iodine solutions (*This Journal,* **22,** 57).

(4) That the preparation and standardization of sodium thiosulfate solutions be studied further.

(5) That the method for the standardization of sodium hydroxide solutions (p. 681) be adopted as official (final action).

(6) That the methods submitted by the associate referee for preparation of standard arsenite solutions (*This Journal,* **21,** 571) be subjected to collaborative study.

(7) That the preparation and standardization of silver nitrate and thiocyanate solutions be studied.

(8) That the method proposed by the associate referee for preparation and standardization of sulfuric acid solutions be adopted as tentative and that collaborative work be done.

(9) That the procedure for preparation of standard hydrochloric acid from constant boiling acid be studied.

(10) That, as amended, the method proposed by the associate referee

* 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.*, 1935.

for the standardization of potassium permanganate solutions be adopted as official (first action).

(11) That the following additional associate referees on standard solutions recommended by the general referee be appointed: sodium thiosulfate, arsenite, constant boiling hydrochloric acid.

INSECTICIDES, FUNGICIDES AND CAUSTIC POISONS

It is recommended—

(1) That pars. **25** and **26,** p. 48, be deleted as this method is not used.

(2) That **3(e),** p. **41,** be amended as suggested by the referee (see p. 77).

(3) That the phrase, "or gravimetrically, weighing the chloride," be deleted, p. 56, **87.**

(4) That the tentative distillation method for the determination of fluorine (p. 46, **18, 19**) be dropped.

(5) That Method I, Procedures I and II, presented by the associate referee for the determination of fluorine in water-soluble or water-insoluble insecticides in the absence of gelatinous silica, boron, and aluminum salts be adopted as tentative.

(6) That Method II presented by the associate referee for the determination of fluorine in water-soluble insecticides in the absence of boron, ferric and aluminum salts, and large quantities of pyrethrin powder be adopted as tentative.

(7) That Method III submitted by the associate referee for the determination of fluorine in insecticides be adopted as official (first action).

(8) That experimental study and collaborative work be conducted on methods for the determination of fluorine next year.

(9) That the mercury reduction method for the determination of pyrethrin I in pyrethrin powder (*This Journal,* **21,** 78) be adopted as official (first action).

(10) That Method 2 for the determination of pyrethrin II in pyrethrin powder (*This Journal,* **22,** 575) be adopted as tentative.

(11) That the crystallization method for the determination of rotenone in derris and cube powder (*This Journal,* **21,** 148) be amended as suggested by the referee (see p. 77), and when so amended that it be adopted as official (first action).

(12) That the method described by the associate referee for the determination of ether extract in derris and cube powders be adopted as official (first action).

(13) That the method for the determination of phenol coefficient (p. 68) be adopted as official (final action).

(14) That, as revised by the associate referee, the mercury reduction method for the determination of pyrethrin I in pyrethrin extracts in mineral oil (*This Journal,* **21,** 78) be adopted as tentative.

FEEDING STUFFS

It is recommended—

(1) That the method described by the Associate Referee on Ash be substituted for the present official method (p. 336, **8**) and made official (first action), and that study be continued.

(2) That the method for the determination of calcium oxide in mineral feeds (p. 347), be adopted as official (first action).

(3) That work be continued on a method for the determination of small quantities of iodine in mineral feeds.

(4) That the qualitative tests for proteins (p. 337) be made official (final action).

(5) That the modified Roese-Gottlieb method for the determination of fat in dried milk products (p. 339, **24**) be made official (first action).

(6) That the methods for the determination of grit in poultry and similar feeds and bone in meat scrap and tankage (p. 347, **42, 43**) be made official (first action).

(7) That the tentative method for the determination of ferrous sulfate, copper sulfate and potassium iodide (**49, 50, 51,** p. 349) be adopted as official (first action).

(8) That the Referee on Feeding Stuffs ascertain whether castor seed is being used in North America as an ingredient of feeding stuffs, and if it is, to continue the study of a method for its detection.

(9) That the tentative method for the determination of manganese (*This Journal,* **21,** 292; **22,** 279), as modified by the associate referee, be adopted as official (first action) and that the study be continued.

(10) That the study of devising standard methods for sampling be continued.

(11) That the method presented by the associate referee for detecting starch in condensed or dried milk products be adopted as tentative.

(12) That a study of methods for the detection of adulteration of cod-liver oil be commenced.

(13) That the paper presented this year on the determination of chlorides in feeding stuffs be studied by the referee and if he deems it advisable that collaborative work be undertaken.

(14) That the study of the determination of lactose in mixed feed be continued and that special consideration be given (1) to the working out of a satisfactory fermentation factor on Fleischmann's small yeast cakes; (2) to determining whether they are uniform in fermentation properties; and (3) to determining which yeast gives the more reliable results—bakers' or the small yeast cakes.

FERTILIZERS

It is recommended—

(1) That the changes in the methods for the analysis of fertilizers suggested by the referee and associate referees be adopted (see p. 75).

(2) That the last sentence, p. 23, **19(g)**, be changed as suggested by the referee.

(3) That the changes made last year in the official methods for the determination of P_2O_5 (*This Journal*, **22**, 70) be adopted as official (final action).

(4) That the distillation method for the determination of water in fertilizers be referred to the Associate Referee on Nitrogen for further consideration of the materials or fertilizers to which it may be applied.

(5) That the Associate Referee on Phosphoric Acid study the fineness of grinding needed for high analysis fertilizer to see if some other degree of fineness should be adopted for general procedure or for special cases.

(6) That, owing to the apparent unsatisfactory results obtained with the MacIntire-Shaw-Hardin method for the determination of available P_2O_5, the method be studied by the Associate Referee on Phosphoric Acid in collaboration with the authors of the method, but that no other collaborative work be undertaken on the method this year.

(7) That the Associate Referee on Nitrogen study the present method for the determination of chlorine in the presence of cyanamid.

(8) That study of the four methods for platinum recovery presented last year (*This Journal*, **22**, 286) be continued.

(9) That diglycol stearate be recognized as a reagent in the preparation of the potash solution and that par. **42**, p. 29, be amended as suggested by the referee (see p. 76) (official, final action, under suspension of the rules, as this is a minor change not affecting the results).

(10) That the words, "and 1 cc of diglycol stearate when necessary to prevent foaming," be added after the word "soln" in line 2, par. **43(a)**, p. 30 (official, final action, under suspension of the rules).

(11) That further collaborative work on a number of samples be done on the investigation of the insoluble residue in potash determinations.

(12) That further study, also collaborative work, be done on the degree of fineness in preparing samples for analysis.

(13) That studies of the solvent action of acid-alcohol and alcohols on K_2PtCl_6 be continued.

(14) That the method for the determination of calcium oxide in stock feeds (p. 347, **44**) be adopted as a tentative method for the same determination in fertilizers, and that bromophenol blue indicator be used.

(15) That the method for lime outlined by the associate referee be studied further and that the determination of calcium by means of the calcium oxalate precipitate from the Bartlett-Tobey method, as modified by J. B. Smith, also be further studied.

(16) That Method 2 outlined by the Associate Referee on Copper be adopted as tentative, and that the electrolytic method for the determination of copper be studied further.

(17) That Method 2 presented by the associate referee for the determi-

nation of zinc be further studied, and collaborative work be done, also that other methods for zinc be investigated.

(18) That further study be made of methods for the determination of sulfur in fertilizers.

(19) That an associate referee be appointed to work on copper and zinc.

(20) That the method, entitled "Magnesia in Water-soluble Compounds" be modified as recommended by the associate referee (*This Journal*, **21**, 77) and adopted as official (first action).

(21) That the Bartlett-Tobey method for the determination of acid-soluble magnesia (*This Journal*, **22**, 270) be amended as recommended by the associate referee and adopted as official (first action).

(22) That the section of the report of the Associate Referee on Magnesia, labeled "Volumetric Modification" be amended as recommended and adopted as a tentative method for the determination of acid-soluble magnesium.

(23) That the study of methods for acid-soluble, water-soluble, and active magnesia in fertilizers be continued.

(24) That the method "Acid-soluble Manganese in Fertilizers and Manganese Salts" (*This Journal*, **21**, 292), be amended as recommended by the associate referee and adopted as official (first action).

(25) That the colorimetric method for the determination of acid-soluble manganese presented last year by the associate referee (*This Journal*, **22**, 279), and again this year, be adopted as tentative.

(26) That the methods for the determination of manganese be further studied.

(27) That on p. 18, **4**, "Moisture," the word "not" be deleted (first word in parentheses).

(28) That the beaker method for the determination of water-insoluble nitrogen (*This Journal*, **22**, 268) be adopted as official (first action).

(29) That the Devarda method (pp. 26–27, **33**) be amended as suggested last year (final action) (see p. 77).

(30) That the copper catalyst suggested in the report on nitrogen for use in the determination of total nitrogen by the Kjeldahl method be studied by the associate referee.

SOILS AND LIMING MATERIALS

It is recommended—

(1) That the procedures presented by the associate referees for replaceable bases in soils devoid of carbonates be adopted as tentative, and that those for available bases in calcareous soils be also adopted as tentative.

(2) That the methods presented by the associate referee for the determination of boron be made tentative and that further studies be made.

(3) That the method submitted by the associate referee for the determination of fluorine be adopted as tentative and that further study be made.

(4) That the method outlined by the associate referee for the determination of *p*H of alkaline soils be adopted as tentative and that work be continued.

(5) That the studies of the exchange bases and exchange capacity of soils be continued.

(6) That the glass electrode be used in the determination of the *p*H values of soils of the humid regions.

(7) That the editorial changes in the methods for soils recommended by the referee be accepted.

PLANTS

It is recommended—

(1) That in the tentative volumetric permanganate method for the determination of reduced copper (**47**, p. 135) the reagent be modified to conform with the preparation of standard solutions (Appendix I).

(2) That the tentative method for determining reduced copper by electrolytic deposition from sulfuric and nitric acid solutions (**48(c)**, p. 136), be deleted.

(3) That Sec. **49** be changed as recommended by the associate referee.

(4) That the tentative method for starch by diastase with subsequent acid hydrolysis (**50**, p. 136) be deleted.

(5) That studies on the determination of hydrocyanic acid, reducing sugars, glucose, fructose, starch, and fructosans be continued.

(6) That the studies on inulin, forms of nitrogen, and sodium and potassium be discontinued for the present.

(7) That studies be inaugurated on zinc, copper, cobalt, and boron.

(8) That studies on less common elements be continued.

(9) That studies be inaugurated on chlorophyl and carotene.

(10) That the methods enumerated by the referee (see p. 79) be adopted as official (final action).

(11) That in line 6, of **12**, p. 124, "5 cc" be changed to "25 cc."

ENZYMES

It is recommended that the study of methods for papain as outlined in last year's report of the committee be continued.

LIGNIN

It is recommended that further study be given to the determination of lignin in plants.

PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

It is recommended—

(1) That further studies be made on the procedure for skinning test, alkali resistance test, and soap resistance test.

(2) That study of the procedure for determining elasticity or toughness of varnish films be continued.

(3) That study of the methods of testing abrasion resistance and hardness of varnish films be continued.

VITAMINS

It is recommended—

(1) That a referee on vitamin C be appointed.

(2) That no associate referee be appointed on technic and details of biological methods, vitamin D carriers.

(3) That the title of the associate referee, "Biological Methods for determination of Vitamin D Carriers," be changed to "Vitamin D for Poultry."

(4) That the title of the associate referee, "Biological Methods for Vitamin B Complexes," be changed to "Vitamin B," and the assignment changed accordingly.

(5) That studies of vitamin A determination be continued.

(6) That study be carried out to determine whether the rat-growth methods of assay of vitamin B content of foods have sufficient accuracy or whether they may be improved by recently described modifications and by application of sulfite treatment.

(7) That the various chemical methods for riboflavin be studied further and that fluorometric methods be considered.

(8) That the Snell-Strong bacteriological technic be further studied.

(9) That further work be done on the biological method of vitamin D carriers to determine how apparent inconsistencies in results can be prevented.

(10) That the method for vitamin D milk be revised as suggested by the associate referee.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES*

By H. J. FISHER (Agricultural Experiment Station, New Haven, Conn.), *Chairman;* A. E. PAUL and W. F. REINDOLLAR

NAVAL STORES

No collaborative work was done this year. It is recommended that the subject be continued.

It is also recommended—

(1) That the methods recommended by the referee be advanced from the status of tentative to that of official (first action) (see p. 78).

(2) That in Sec. 2, p. 75, Acid Number, provision be made for the use

* 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.*, 1935.

of denatured alcohol, known as "Formula 30," and that the words "and cool to room temperature," line 3, be deleted.

(3) That the first four lines of the saponification number method be changed as suggested by the referee (see. p. 78).

(4) That the method for the determination of toluol-insoluble matter, 5, 6, p. 76, be changed as suggested by the referee (see p. 78).

(5) That Sec. 18, p. 82, be changed as suggested by the referee (see p. 78).

(6) That Sec. 19 be changed as suggested by the referee (see p. 78).

RADIOACTIVITY

It is recommended that the method for the determination of radioactivity be revised in accordance with modern practice. It is also recommended that a general referee on radioactivity be appointed and that the separate associate refereeships on quantum counter and gamma ray scope be discontinued.

COSMETICS

No work was done on this subject. It is suggested that a general referee on the subject be appointed, and also associate referees on the following subjects:

(1) Hair preparations (tonics, bleaches, shampoos, wave lotions).

(2) Colored make-up preparations (hair dyes, lipsticks, rouges, eyebrow and eyelash preparations, mascara).

(3) Facial preparations (face powders, creams, and lotions; shaving creams, sticks, and powders).

(4) Dentifrices and mouth washes.

(5) Miscellaneous (depilatories, anti-wrinkle preparations; wart, mole, and corn removers).

DRUGS

MICROCHEMICAL TESTS FOR ALKALOIDS

It is recommended—

(1) That the microchemical tests for physostigmine and stovaine proposed by the associate referee be adopted as tentative.

(2) That the status of the microchemical tests for the following alkaloids be advanced from official (first action) to official (final action):

Aconitine	Codeine	Pilocarpine
Apomorphine	Ephedrine	Procaine
Arecoline	Ethylhydrocupreine	Quinidine
Atropine	Ethylmorphine	Quinine
Benzylmorphine	Homatropine	Scopolamine
Brucine	Hydrastine	Sparteine
Caffeine	Hydrastinine	Strychnine
Cinchonidine	Hyoscyamine	Theobromine
Cinchonine	Nicotine	Theophylline
Cocaine	Papaverine	Yohimbine

(3) That the associate refereeships on microchemical tests for alkaloids and synthetics be combined as one, and that dilaudide be one of the substances studied.

MICROCHEMICAL TESTS FOR SYNTHETICS

It is recommended—

(1) That the status of the microchemical tests for the following synthetics be advanced from official (first action) to official (final action):

Acetanilid	Barbital	Neocinchophen
Acetophenetidin	Benzocaine	Phenobarbital
Acetylsalicylic acid	Benzoic acid	8-Hydroxyquinoline
Aminopyrine	Cinchophen	Pyridium
Amytal	Dinitrophenol	Salicylic acid
Antipyrine	Methenamine	Triethanolamine

(2) That further study of hair dye intermediates be transferred to the Referee on Coloring Matters in Drugs and Cosmetics.

DAPHNIA METHODS

It is recommended that this subject be dropped.

ERGOT ALKALOIDS

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

GUAIACOL IN MIXTURES

No report was received from the associate referee this year. It is recommended that this subject be dropped.

BIOLOGICAL TESTING

No report was submitted. It is recommended that the subject be reassigned.

IODINE OINTMENT

A verbal report was received from the associate referee. He suggested that a method for iodide iodine specifying an adsorption indicator be investigated. It is recommended that the subject be continued.

ACETOPHENETIDIN, ACETYLSALICYLIC ACID, AND SALOL

The associate referee submitted two methods for the separation of these substances to collaborative study. Good results were obtained with one method (Method I). It is recommended that the method be adopted as tentative, and that the subject be closed.

AMINOPYRINE AND PHENOBARBITAL IN MIXTURES

No report was submitted. It is recommended that the subject be discontinued.

ELIXIR OF TERPIN HYDRATE AND CODEINE

The associate referee has modified the method previously studied to eliminate points of error. The modified method gave excellent collaborative results. It is recommended that the method be adopted as tentative and that the subject be closed.

OINTMENT OF YELLOW MERCURIC OXIDE

The associate referee studied the U.S.P. XI method in detail. It is recommended that his study be referred to the U.S.P. Revision Committee, and that this subject be dropped.

RHUBARB AND RHAPONTICUM

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

THEOPHYLLINE SODIUM SALICYLATE

The associate referee further studied the assay of this product, but no collaborative work was done. It is recommended that the subject be continued.

MANDELIC ACID MIXTURES

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

PHYSOSTIGMINE SALICYLATE

The associate referee submitted a method for the determination of this product to collaborative study. Results of collaborators were mostly low. It is recommended that the subject be continued.

SEPARATION OF ACETANILID AND SALOL

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

ARECOLINE HYDROBROMIDE

The associate referee made a preliminary study of this topic. It is recommended that the subject be continued.

BENZEDRINE

Preliminary studies of this subject were made by the associate referee. It is recommended that the subject be continued.

PLASMOCHINE

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

HYDROXYQUINOLINE SULFATE

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

PEPSIN

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

IPECAC AND OPIUM POWDER

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

NICOTINIC ACID

The associate referee devised a sublimation method for the determination of nicotinic acid in tablets. The method was submitted to collaborative study, and excellent results were obtained. It is recommended that the method be adopted as tentative and that the subject be closed.

EPHEDRINE IN JELLIES

Some difficulty has been experienced by certain analysts in applying the method for ephedrine in inhalants (p. 557, 42) to products of this type. The associate referee investigated this matter, and found that correct results for ephedrine can be obtained if no heat is used in evaporating the ether solution of the alkaloid. It is accordingly recommended that the method be amended by the deletion of the phrase, "on a steam bath with moderate heat," from the next to last line of page 557, and that the subject be closed.

PURIFICATION OF CAFFEINE IN PLANT EXTRACTIVES

This subject was studied collaboratively. It was found that a slight modification of the present method for the purification of caffeine would eliminate the losses previously experienced. It is accordingly recommended that the method for the determination of caffeine (p. 545, 7(a), line 1) be amended by the addition after the word "add" of the phrase "1 cc of 9*N* H_2SO_4 and."

ASSAY OF OINTMENT OF RED MERCURIC IODIDE

Rupert Hyatt submitted a contributed paper on this subject. He has worked out a method that gives good results. It is recommended that this and other methods be studied with a view to the adoption of a uniform method for ointments of mercury and its compounds.

DETERMINATION OF PROCAINE

Matchett and Levine submitted a contributed paper on this subject. They have worked out a new method for procaine which is not affected by the presence of heroin, codeine, or cocaine. It is recommended that this method be adopted as a tentative method for procaine.

CHANGES IN STATUS OF METHODS

In accord with the recommendation of the Referee on Drugs, the Committee recommends that the following methods be advanced from official (first action) to official (final action):

Acetanilid and Caffeine (p. 543, 5).

Acetanilid, Caffeine, and Codeine (p. 545, 8).

Acetanilid, Caffeine, and Quinine (p. 546, 10).

Acetanilid, Caffeine, Quinine, and Morphine (p. 546, 12).

Acetanilid and Sodium Salicylate (p. 547, 14).

Aloin (p. 567, 75).

Antipyrine and Caffeine (p. 553, 32).

Apomorphine in Tablets (p. 565, 67).

Atropine in Tablets (p. 555, 37).

Barbital and Phenobarbital (applicable in presence of stearic acid) (p. 582, 113).

Cocaine, Method I (p. 576, 96).

Dinitrophenol and its Sodium Compound (*This Journal*, 20, 596).

Emetine Hydrochloride in Tablets (p. 554, 35).

Ether (p. 584, 120).

Homatropine in Tablets (*This Journal*, 21, 95; 22, 100).

Iodoform and Iodoform Gauze (p. 594, 148, 150).

Menthol (p. 571, 83).

Morphine in Sirups (p. 586, 125).

Phenolsulfonates (p. 597, 161).

Thymol in Antiseptics (p. 572, 85).

In accord with the recommendation of the Referee on Drugs, it is recommended that the following methods be advanced from tentative to official (first action):

Calomel in Calomel Ointment (p. 595, 153).
Hypophosphites in Sirup (p. 593, 145).
Monobromated Camphor, Method II (p. 561, 55).
Salicylic Acid in Presence of Other Phenols (p. 570, 81).
Tetrachlorethylene in Mixtures (p. 581, 109).

IPOMEA AND JALAP

As it is the policy of the Association not to retain a method for a product for which an assay is provided in the U. S. Pharmacopoeia or National Formulary, the Committee considers that the A.O.A.C. methods (p. 566–7, 72, 73) should be deleted for the present, and so recommends.

METHYLENE BLUE

The U. S. Pharmacopoeia has recently adopted a perchlorate method for the assay of this substance. This method does not include a separation of the drug from mixtures. It is recommended that the official A.O.A.C. iodine method (p. 559, 48) be retained for the present, but that an associate referee be appointed to study the possibility of combining the A.O.A.C. method of separation with the perchlorate method of determination.

SWELLING FACTOR OF PSYLLIUM

Since the National Formulary has adopted a method for this determination, it is recommended that the present tentative A.O.A.C. method (p. 587, 126) be deleted.

IODOFORM OINTMENT

Through an oversight last year this method (p. 594, **149**) was not advanced to official (first action) as were the methods for iodoform and iodoform gauze. It is recommended that this method be made official, (final action) under suspension of the rules.

SANTONIN

Last year the method for santonin in mixtures (p. 587, **129**) was made official (first action), but the method for santonin in tablets (**128**) was left as tentative. In the opinion of the referee and the former associate referee, these two methods should be placed under the one heading "Santonin in Mixtures" as Methods II and I, respectively. In order that this may be possible, and as both methods are sound, it is recommended that both methods be made official (final action), the latter under suspension of the rules.

PYRAMIDON (AMINOPYRINE)

As it has been found by several analysts that sulfuric acid is more effective than hydrochloric in holding aminopyrine in solution, it is recommended that the official method for pyramidon (aminopyrine) (p. 573, **87**) be amended by substituting "H_2SO_4" for "HCl," this amendment to be made official (final action) under suspension of the rules.

CAMPHOR

Last year the official method for the determination of camphor (p. 560, **51**) was amended (first action) by the insertion between the title and the text of the parenthetical phrase "Not applicable to synthetic camphor." It is recommended that the amendment be made official (final action).

BARBITAL AND PHENOBARBITAL

Last year the official method for the determination of barbital and phenobarbital (p. 582, **112**) was amended (first action) by the addition at the end of par. **112** of the following expression: "Determine the melting point to check the purity of the residue."

It is recommended that this amendment be now made official (final action).

GENERAL RECOMMENDATION

The Committee has given earnest consideration to the fact that the work in the field of drugs has grown to such an extent that it now covers a very large proportion of the activities of the Association. The opinion is that no one referee should be expected to bear the entire burden of supervising so extended a field. It is therefore recommended that in place of one general referee on drugs, four separate refereeships be set up covering the following topics:

(1) Synthetic Drugs.
(2) Vegetable Drugs and Their Derivatives.
(3) Drug Bioassays.
(4) Miscellaneous Drugs.

NEW SUBJECTS

It is recommended that the following subjects be studied:

(1) Aminopyrine, acetophenetidin, and caffeine.
(2) Ethyl aminobenzoate (benzocaine).
(3) Quinine ethyl carbonate (euquinine).
(4) Sulfapyridine.
(5) Theobromine and phenobarbital.
(6) Magnesium trisilicate.
(7) Mercury ointments.
(8) Methylene blue.
(9) Emulsions.
(10) Compound ointment of benzoic acid.
(11) Elixir of three bromides.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES*

By G. G. FRARY (State Chemical Laboratory, Vermillion, S. D.), *Chairman;* W. B. WHITE and J. O. CLARKE

CANNED FOODS

It is recommended—

(1) That the method for the determination of chlorides in tomato products described by the associate referee be adopted as tentative with the explanatory caption "(Rapid Method)."

(2) That the official method for the determination of total chlorides in tomato juice (*This Journal*, **20**, 78; **22**, 88) be dropped (first action).

(3) That the tentative method for the determination of total solids in tomato products (p. 499, **16**) be clarified as recommended by the associate referee and subjected to further study.

(4) That the official method for preparation of sample of vegetables and vegetable products (p. 497, **2**) be clarified by the incorporation of the editorial changes suggested by the referee, and given the explanatory caption "(Applicable to canned products only)"; and that the method be incorporated by reference under a similar caption under "Fruit and Fruit Products" (p. 319, **2**).

(5) That the tentative method for physical examination of vegetables and vegetable products (p. 497, **1**) be given the explanatory caption "(Applicable to canned products only)."

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* 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.*, 1935.

(6) That the obvious error in the method for the determination of moisture in vegetables and vegetable products (p. 497, **3**) be corrected by the substitution of "XXVII, 2" for "XXVII, 5."

(7) That the clarifying editorial change in the method for yeasts and spores (p. 501, **29**) recommended by the referee be made.

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

(9) That the feasibility of harmonizing the method for the determination of ash in tomato products (p. 499, **20**), with that for ash in vegetables and vegetable products (p. 497, **4**), be investigated.

COFFEE AND TEA

It is recommended—

(1) That the tentative method for the determination of volatile oil in tea (p. 196, **40**) be dropped.

(2) That studies be made of methods for the determination of chlorogenic acid in coffee.

DAIRY PRODUCTS

It is recommended—

(1) That the stirrer method recommended by the associate referee for preparation of butter samples be adopted as tentative.

(2) That the tentative method for the determination of moisture, fat, and salt in butter (p. 288, **79** and **80**) be dropped.

(3) That rapid methods for the direct determination of fat in butter be studied.

(4) That studies of methods for the determination of fat in malted milk be continued and extended to include methods involving more complete hydrolysis of non-fat constituents.

(5) That studies of methods for the determination of lactic acid in dried milk and skim milk be continued.

(6) That studies of methods for the detection of neutralizers in dairy products be continued, with particular attention given to the ratio between titrable acidity and lactic acid.

(7) That the tentative method for mold mycelia in butter (*This Journal*, **22**, 76) be extended to include the alternative staining procedure recommended by the associate referee.

(8) That studies of methods for the determination of the degree of pasteurization of milk be continued.

(9) That studies of methods for the detection of the use of unpasteurized cream in the manufacture of butter be continued.

(10) That studies be made of methods for the determination of dextrose in sweetened condensed milk.

(11) That studies be continued on methods of isolating fat from cheese for the determination of fat properties and constants.

(12) That studies be made looking toward the unification of methods for the determination of total solids and ash in milk and evaporated milk.

(13) That the editorial changes in the method for the determination of fat in milk (p. 267, 19) suggested by the referee be made.

(14) That the Babcock method for the determination of fat in milk (p. 268, 20) be studied with a view to placing a tolerance range on the revolutions per minute of the various sizes of centrifuge.

(15) That studies of methods for the determination of casein in malted milk be continued.

(16) That studies on methods for the detection of decomposition in dairy products be continued.

(17) That studies of methods of analysis of frozen desserts be continued.

(18) That the associate referee's suggestion to delete the clarifying definition of the temperature of mixing the samples (*This Journal*, **21**, 84) in the official method for the determination of fat in butter be accepted, and that the method be retained as given on p. 288, **76.**

(19) That studies on methods for the clarification of milk for the optical determination of lactose be continued and broadened to include correction for volume of the precipitate.

(20) That the method described by the Referee on Gums in Foods for the detection of gums in soft curd cheese be adopted as tentative.

EGGS AND EGG PRODUCTS

It is recommended—

(1) That the tentative method for the determination of unsaponifiable matter (p. 300, 13) be dropped.

(2) That studies of methods for the determination of cholesterol and fat be continued.

(3) That the amended method for the determination of chlorine (*This Journal*, **22**, 77) be made official (final action).

(4) That the amended method for the determination of dextrose and sucrose (*This Journal*, **22**, 77) be made official (final action).

(5) That studies of methods for the determination of glycerol be continued.

(6) That studies of chemical methods for the detection of decomposition be continued.

FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the tentative methods for the determination of ash, salt, and total nitrogen (*This Journal*, **21**, 86) be made official (first action).

(2) That studies of methods for the determination of total solids and ether extract be continued.

(3) That studies be made of the applicability of methods for the deter-

mination of other substances in meat products to fish and other marine products.

GUMS IN FOODS

It is recommended—

(1) That the method for the detection of gums in mayonnaise and French dressing (*This Journal*, **22**, 607) be adopted as tentative.

(2) That the tentative method for detection of gums in cheese (p. 295, **106**) be dropped.

(3) That studies of methods for the detection of gums in frozen desserts and in starchy foods be continued, and extended to other foods as opportunity permits.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That the title of the tentative method for added water in sausage and similar meat products (p. 353, **3**) be changed to read, "Added Water in Sausage."

(2) That action on the modification of the official method for creatin (p. 360, **31**) recommended by the referee be postponed until supporting data are available.

(3) That the tentative method for the determination of nitrogen in gelatin (p. 367, **62**) be modified to conform to the method for meat (p. 354, **8**).

(4) That studies of methods for the detection and determination of dried skim milk and soy bean flour be continued.

METALS IN FOODS

It is recommended—

(1) That studies be continued on methods of sample preparation of those products wherein the arsenic is tenaciously held.

(2) That the iodine titration, gold or silver sol, and the molybdenum blue colorimetric methods for the determination of arsenic be further studied as possible substitutes for the Gutzeit method.

(3) That in the studies of methods for the determination of antimony and of arsenic, special attention be given to the separation of micro quantities of these elements occurring simultaneously in organic or biological material.

(4) That studies on micro methods for the determination of copper be continued.

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

(6) That the tentative method for the determination of lead (p. 381, **16(a)**; 387, **25(a)**; 388, **26(a)**) be modified as suggested by the referee.

(7) That studies on the determination of lead be continued.

(8) That the editorial change in the official rapid method for lead (pp. 391–3, 31 and 32) suggested by the referee be made.

(9) That the tentative method for the determination of mercury (pp. 393–5, 34, 35, and 36) be changed as recommended by the associate referee.

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

(11) That the method proposed by the Associate Referee for the determination of zinc be adopted as tentative, and that work be continued.

(12) That the method developed by the associate referee for determining hydrocyanic acid be subjected to further study.

MICROBIOLOGICAL METHODS

It is recommended—

(1) That the methods for frozen egg products (*This Journal*, **22**, 625), as revised by the associate referee, be adopted as tentative.

(2) That the methods for canned vegetables presented by the associate referee be adopted as tentative.

(3) That the methods recommended by the associate referee for detecting and estimating the number of thermophilic bacteria in sugar be adopted as tentative.

(4) That studies be continued on methods for canned vegetables, canned tomatoes, canned fruits, canned fishery products, canned meats, sugar, and eggs and egg products.

OILS, FATS, AND WAXES

It is recommended—

(1) That the modified Kaufmann method for determining the thiocyanogen number of fats and oils (*This Journal*, **21**, 87) be studied collaboratively with a view to its adoption as official.

(2) That studies be made on the application of the official method for refractometric determination of oil in flaxseed (*This Journal*, **20**, 74) to other oil seeds.

(3) That studies on the Polenske method be continued.

(4) That the precautionary statement suggested by the referee be inserted in the method for the determination of cholesterol and phytosterol (p. 418, 33).

(5) That the methods for sampling and analyzing cottonseed (p. 423) be changed as suggested by the referee and adopted as official (final action).

(6) That the omission of the caption "Official" in the methods for cottonseed (p. 423) be rectified.

(7) That methods for the determination of olive oil in admixture with other oils be developed.

SPICES AND CONDIMENTS

It is recommended—

(1) That studies on the determination of total solids in vinegar be continued with special reference to vinegars high in solids.

(2) That studies on the determination of total phosphoric acid in vinegar be continued and extended to include soluble and insoluble phosphoric acid.

(3) That studies on the detection of caramel in vinegar be continued.

(4) That the "oxygen value" mentioned by the referee be studied in regard to its usefulness in differentiating between distilled vinegar and commercial acetic acid.

(5) That the tentative method for preparation of samples of salad dressing (p. 454, **43**) be studied collaboratively in connection with the studies recommended in (6) below, with a view to adoption as official.

(6) That the tentative methods for the determination of sugars, fat, composition, and identification of oil of salad dressings be studied collaboratively.

(7) That the tentative method for the determination of volatile oil in spices (p. 444, **16**) be made official (final action) for marjoram and sage, and be further studied as to its application to other spices.

(8) That studies of methods for the determination of starch in mustard flour and prepared mustard be continued.

(9) That the official method for volatle oil in mustard seed (p. 450, **25**) be studied as to its application to different types and to other mustard products.

(10) That studies be made of methods for the direct determination of moisture in spices.

(11) That studies be made on the method for the determination of ash in spices (p. 445, **3**) and in prepared mustard (p. 452, **34**) with a view to fixing the temperature of ignition, and that studies be initiated on methods, other than ashing, for measuring soil contamination.

(12) That an editorial change suggested by the associate referee be made in the method for the determination of acid-insoluble ash of spices (p. 445, **5**).

(13) That the corrections suggested by the referee be made in the method for the determination of reducing sugars before inversion (Salad Dressings, p. 454, **45**), (see p. 87).

(14) That the tentative method for reducing sugars after inversion (Salad Dressings, p. 454, **46**) be amended as recommended by the referee (see p. 87).

(15) That the tentative method for determining the iodine number of paprika oil (p. 450, **26**) be made official (first action) and that the words "Qualitative test for presence of foreign oil" be substituted for "Indication of presence of olive oil—qualitative test" in the heading.

(16) That studies be made of methods for the determination of salt in prepared mustard.

REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES*

By W. C. JONES (Department of Agriculture, Richmond, Va.), *Chairman;* J. W. SALE, and J. A. LECLERC

SUGAR AND SUGAR PRODUCTS

It is recommended—

(1) That the method for the determination of sucrose and raffinose by polarization before and after treatment with two enzymes (p. 474, **24,** and **25**) be made official (final action).

(2) That Somogyi's modification of Shaffer and Hartman's microchemical method for reducing sugars be adopted as tentative.

(3) That study of drying, densimetric, and refractometric methods be continued.

(4) That studies on the determination of moisture in honey be continued.

(5) That the work on methods for determining acetyl-methyl carbinol and diacetyl in food products be continued.

(6) That the study of maple flavor concentrates and imitations be continued.

(7) That the work on methods for determining the so-called unfermentable reducing substances of molasses be continued.

(8) That work be done on sucrose in molasses.

(9) That the procedure for ash (p. 465, **8, 9, 10**) be changed as recommended by the associate referee.

(10) That the work on refractive indices of invert sugar solutions and the change in refractive indices with change of temperature in such products as invert sugar solutions, table sirups, etc., be continued.

(11) That the method presented by the referee for electrolytic deposition from nitric acid solution be adopted as tentative and replace **42,** p. 481.

(12) That a new column be inserted in Munson and Walker's reducing sugar table (p. 629, **9**) comprising the copper equivalents of pure levulose.

(13) That a column of copper equivalents of pure invert sugar be substituted for the values now given in Munson and Walker's table (p. 629, **9**).

(14) That the conversion factors of the different saccharimeter scales be revised as recommended by the referee.

(15) That the volumetric thiosulfate method (**39,** p. 480) be revised as recommended by the referee.

(16) That the volumetric permanganate method (**41,** p. 480) be revised as suggested by the referee.

* These recommendations, submitted by Subcommittee D, were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis, A.O.A.C.*, 1935.

(17) That Tables 6 and 7, **XLII**, be revised as suggested by the associate referee.

(18) That in both methods **3** and **4**, pp. 462–463, the pressure of "100 mm of Hg" be changed to read "50 mm of Hg."

WATERS, BRINE AND SALT

It is recommended—

(1) That the thorium nitrate titration method for the determination of fluorine, referred to in the referee's report, be adopted as tentative.

(2) That the tentative method for the determination of fluorides (p. 508, **22–26**, inclusive, be dropped.

(3) That studies on moisture in effervescent salts be continued.

(4) That studies on boron in water be continued.

CACAO PRODUCTS

It is recommended—

(1) That the changes submitted by the referee to be made in Pars. **9, 10, 12,** and **26** be accepted (see p. 82).

(2) That the method for the determination of shell in cacao nibs (cocoa nibs) described in the report of the referee be adopted as a tentative method.

(3) That further collaborative work be done on the method for the determination of pectic acid in cacao products.

(4) That collaborative work be done on the rapid method for determination of milk protein in milk chocolate described in last year's report (*This Journal*, **22**, 600).

(5) That methods for determination of lecithin in cacao products be studied.

(6) That methods for the determination of the chocolate content of sweet and milk chocolate be developed.

ALCOHOLIC BEVERAGES

MALT BEVERAGES, SIRUPS, AND EXTRACTS, AND BREWING MATERIALS

It is recommended—

(1) That the study of methods for the analysis of malt adjuncts be continued.

(2) That the tentative method for the determination of diastatic power (p. 158, **46(b)**) be revised as suggested by the associate referee (see p. 80).

(3) That the changes suggested by the associate referee be made in the methods for beer **(XIV)**, (see p. 80).

(4) That the method submitted by the associate referee for the determination of total acidity be made tentative.

(5) That the colorimetric methods for Fe, Cu, and Sn in beer recommended by the associate referee be studied.

(6) That the tentative method for the determination of pasteurization be deleted.

(7) That the procedures recommended by the associate referee for H-ion concentration be adopted as tentative (see p. 80).

(8) That the method for the determination of end fermentation as increase in the degree of fermentation submitted by the associate referee be adopted as tentative.

(9) That the subject of hops be studied.

(10) That the Associate Referee on Beer study collaboratively the following tentative methods relating to beer: (a) Extract in Original Wort; (b) Real Degree of Fermentation; (c) Total Acid; (d) Dextrin; (e) Direct Polarization; (f) Pasteurization; (g) Chlorides; and (h) H-ion Concentration.

(11) That the viscometric method outlined by the associate referee last year for the determination of proteolytic activity of malt (*This Journal*, **21**, 160) and the edestin titration method (*Wochschr. Brau.*, **53**, 297 (1936)) be further studied.

(12) That special study be made of the diastatic activity of malt.

(13) That the collaborative study of sulfur dioxide in beer and ale be continued and also be extended to include this same determination in wines.

(14) That the method for sulfur dioxide (p. 152, **21**) be deleted, and that the method described in the associate referee's report be made official (first action).

(15) That the tentative manometric method for the determination of CO_2 in beer be further studied collaboratively with the object of making it official (first action).

(16) That the selenium method for digestion of sample for protein in beer, suggested by the associate referee in 1939, be studied.

(17) That the study of methods for the proteolytic activity of malt be continued.

(18) That **19–20**, p. 151, "Carbon Dioxide—Tentative" be deleted.

(19) That in **51**, p. 161, line 2, and in **55**, line 7 from bottom, "gypsum plate," be changed to read, "into the cavities of a porcelain plate."

(20) That the formula at the end of **21**, p. 161, be corrected.

(21) That to **56**, p. 162, "See **53**, **54**, and **55**," be added.

(22) That study on heavy metals in beer be continued.

(23) That study on malt extract in malt be continued.

WINES

It is recommended—

(1) That the modified Peynaud procedure for volatile acidity of wines (*This Journal*, **22**, 210) be adopted as tentative after it has been revised as directed by the associate referee.

(2) That the effect of lactic acid on the volatile acidity of wines be reinvestigated for the various types of apparatus now in use.

(3) That Method II (official), p. 167, for total volatile acidity in wines, be changed as suggested by the associate referee.

(4) That Sec. **25**, p. 167, be changed as suggested by the associate referee.

(5) That the notation "(Especially adapted to wine)," **33**, p. 441, be deleted.

(6) That the method for sulfurous acid in wine described by the associate referee be made tentative and that the method be further studied.

DISTILLED LIQUORS

It is recommended—

(1) That the methods for acids in distilled liquors presented by the associate referee be adopted as official (first action).

(2) That study on methods for the analysis of whiskey and rum and on denaturants in distilled spirits be inaugurated.

(3) That the method for aldehydes presented by the associate referee last year (*This Journal*, **22**, 73) be adopted as official (first action).

(4) That the method for the determination of benzaldehyde in cordials (p. 183, **55**, **56**) be adopted as official (first action).

(5) That the method for the determination of volatile esters in cordials (p. 181, **46**) be adopted as official (first action).

(6) That the method for gamma-undecalatone, qualitative (p. 181, **47**) be adopted as official (first action).

(7) That the method for total solids "From the residue of the dealcoholized sample" (p. 180, **37(c)**) be adopted as official (first action).

(8) That the studies on wood alcohol in distilled spirits and on detection of adulteration of distilled spirits be continued.

(9) That on p. 173, top, the 2nd paragraph be omitted and the following substituted: "It is preferable to use ground-glass connections throughout."

(10) That on pp. 173, 174, **19**, the Trillat method—official; Method **20**, the Riche and Bardy method—official; and **21** and **22**, the modified Denigès method, be deleted.

(11) That the method submitted by the associate referee for the determination of methyl alcohol be substituted for the modified Denigès method, **21**, **22**, p. 174.

(12) That the modified Marsh test (p. 179, **33**) be made official (first action).

(13) That Sec. **8**, p. 170 (Acidity—official) be followed by No. 9, Fixed Acidity—Tentative, and No. 10, Volatile Acidity—Tentative, and that the three methods be arranged as suggested by the associate referee.

(14) That the following changes be made in the following methods:

P. 180, **37(c)**.—Change "residue" to "refractive index."

P. 181, **46**, line 1.—Change "proceed" to "steam distil."

P. 182, **51.**—Change to read, "Using ash obtained under **50**, proceed as directed under **XXXIV, 12.**"

P. 182, **52.**—Change to read, "Using soluble ash obtained under 51, proceed as directed under **XXXIV, 13.**"

P. 182, **53.**—Change to read, "Using insoluble ash obtained under **51**, proceed as directed under **XXXIV, 14.**"

FOOD PRESERVATIVES AND SWEETENERS

It is recommended—

(1) That the qualitative test for saccharin be studied further with a view to learning its applicability (a) to other foodstuffs, and (b) in the presence of interfering substances.

(2) That the work on the Illing method for the determination of benzoate of soda, which was found to be suitable for sausage, be continued with respect to its suitability for other food products.

COLORING MATTERS IN FOODS

It is recommended—

(1) That collaborative work be continued on the quantitative determination of ponceau SX in the presence of ponceau 3R.

(2) That investigational work be continued on the quantitative separation and determination of tartrazine and sunset yellow FCF in mixtures.

(3) That investigational work be undertaken to separate and determine quantitatively mixtures of light green SF yellowish, brilliant blue FCF, and fast green FCF.

(4) That method **16(b),** p. 245, be changed as suggested by the referee (see p. 84).

(5) That collaborative work on analytical methods for the coal-tar colors certifiable for use in foods be conducted.

(6) That the Association authorize the renaming of colors used in foods, drugs, and cosmetics to accord with the nomenclature used in the Federal Food, Drug, and Cosmetic Act.

(7) That the methods for the determination of arsenic and lead be revised to conform to the changes made in these methods under Chapter **XXIX.**

FRUIT AND FRUIT PRODUCTS

It is recommended—

(1) That the study of electrometric titrations of acids be continued.

(2) That the study of inactive malic, isocitric, citric and lactic acids in fruit products be continued.

(3) That the study of polarimetric methods for fruit products be continued.

(4) That the changes recommended by the referee to be made in the methods for the analysis of preserves and jams (*This Journal*, **22,** 78) be adopted as official (final action).

(5) That the colorimetric method for determination of P_2O_5 in fruits and fruit products be made tentative and that the method be further studied collaboratively.

(6) That the changes in the methods for ash (p. 321, **9,**) suggested by the associate referee be adopted (see p. 85).

(7) That the study of ash in fruit products be discontinued.

(8) That studies be inaugurated on the following subjects: gravimetric determination of P_2O_5, sodium and chlorine, and potassium.

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That work on the determination of gylcerol, vanillin, and coumarin in vanilla and imitation vanillas be continued, with special reference given to automatic extraction of vanillin and coumarin and the spectrophotometric method for coumarin.

(2) That the procedure for quantitative precipitation of β-ionone as *m*-nitrobenzhydrazide (*This Journal*, **22,** 383) be adopted as official (first action), and published under the heading, "Favoring Extracts: Beta-Ionone, Quantitative. (Applicable to pure solutions of not over 100 mg of β-ionone in 5 cc of alcohol)," and that collaborative work be done.

(3) That the procedure for β-ionone in raspberry flavors (*This Journal*, **22,** 386) be adopted as official (first action) for beverage concentrates, after the addition of precautions relating to volume of distillate and extraction procedure as described by the referee and that collaborative work be done.

(4) That the changes submitted by the referee be made in the text of Chapter **XIII.**

(5) That the quantitative method for determination of isopropyl alcohol in extracts, described in the report of the associate referee this year and in *This Journal*, **22,** 594, be submitted to collaborative study.

(6) That the method for determination of β-ionone when 1–10 mg is present (*This Journal*, **22,** 691) as applied to beverages be submitted to collaborative study.

(7) That collaborative study be made of the application of the Ripper method for aldehydes in spirits to lemon oils and extracts.

(8) That collaborative study be made of the application to emulsion flavors of the official steam distillation method for lemon, orange, and lime oils in vegetable and mineral oils.

BAKING POWDER

It is recommended—

(1) That the methods for the quantitative determination of total tartaric acid, cream of tartar, and free tartaric acid in tartrate baking powders (*This Journal*, **22,** 74) be adopted as official (final action).

(2) That the present official method for the determination of total tar-

taric acid (p. 186, **13**) be deleted (final action under suspension of the rules).

CEREAL FOODS

It is recommended—

(1) That the changes submitted by the referee be made in Chapter XX, Cereal Foods (see p. 83).

(2) That the magnesium-acetate method (*This Journal*, **20**, 69), for ashing cereal products be made official (final action), and be published in place of the present tentative method (p. 207, **6**, 7).

(3) That the method outlined by the associate referee for the determination of H-ion concentration, with sulfonphthalein indicator, be substituted for the present method (p. 208, **14**) and made official (first action) for flour, bread, other baked products, and macaroni, and that further work be done, especially with electrometric measurement.

(4) That further study be given to methods for starch in raw and cooked cereals.

(5) That the modification in the methods proposed by the associate referee for the determination of fat acidity in grain, flour, corn meal, and whole wheat flour be made tentative and that further work be done.

(6) That the modified method proposed by the associate referee for the determination of sugar in flour be made official (first action) and that the tentative method for sugars (p. 209, **15**), in so far as it relates to cereal products, be deleted.

(7) That the associate referee continue the study of the baking test for soft wheat flour.

(8) That the quick ashing method submitted by the associate referee for the determination of chlorine in fat extracted from flour be made official (first action).

(9) That further studies be made on methods to determine benzoyl peroxide in flour.

(10) That further studies be made to determine the carotenoid pigments in flour, and that procedures **34**, **35**, **36**, and **37**, pp. 214–15 be deleted.

(11) That the tentative method for the determination of CO_2 in self-rising flour (p. 208, **9**), as modified by the associate referee, be adopted as official (first action), and that further work be done.

(12) That the lactose procedure for the determination of milk solids in bread be studied collaboratively.

(13) That the study of a method for the determination of butter fat content of bread, based upon direct saponification and distillation of bread without previous extraction of fat be discontinued.

(14) That further study be made to improve the "fat method" for the determination of milk solids in bread (p. 222, **51**).

(15) That study be continued on methods to determine cold-water extract of flour.

(16) That the study of ergot in flour be discontinued.

(17) That the study of the proteolytic activity of flour as recommended by the associate referee be continued.

(18) That the associate referee continue the study of color measurements of flour and bread.

(19) That the method for the qualitative detection of soya flour in cereal products (*This Journal*, **17**, 324) be adopted as tentative, with the change in indicator from bromophenol to bromothymol blue.

(20) That the study of methods for the determination of cellulose in whole wheat flour products be continued along the lines suggested by the associate referee.

(21) That the method studied by the associate referee for the determination of original ash in phosphated and self-rising flour be adopted as official (first action) and that flour work be continued.

(22) That the following official methods for flour be adopted as tentative for rye, oats, corn, and buck wheat and their products:

MOISTURE, p. 206, **2.**
ASH, p. 207, **5.**
FAT, p. 208, **10.**
FIBER, p. 208, **12.**
PROTEIN, p. 209, **16,** using the factor 6.25.

It is also recommended that further work be done.

(23) That the same study be made with rice and barley products.

(24) That the following official methods for bread appearing under Cereal Foods be adopted as tentative for baked products other than bread not containing fruits:

TOTAL SOLIDS, p. 222, **51, 52.**
ASH, p. 224, **56.**
PROTEINS, p. 224, **58.**
FAT, p. 224, **59.**
FIBER, p. 224, **60.**

(25) That collaborative study be continued with these methods to determine their applicability to other cereal products, such as cake, breakfast foods, crackers.

(26) That an associate referee be appointed to study methods for the determination of moisture in self-rising flour, pancake, waffle and doughnut flours.

(27) That study of the method for determination of NaCl-free ash in baked products and macaroni be discontinued.

(28) That studies be continued on methods for the identification of the raw materials used in the manufacture of macaroni.

(29) That studies on the sterol content of flour be continued.

MICROCHEMICAL METHODS

It is recommended that study be continued.

CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FIFTY-FIFTH ANNUAL MEETING, OCTOBER 30, 31, AND NOVEMBER 1, 1939*

The detailed directions are not given in this report as usual because they will appear very shortly in the 5th (1940) Edition of *Methods of Analysis, A.O.A.C.*

I. SOILS

(1) The procedures presented by the associate referee for determining replaceable bases in soils devoid of carbonates were adopted as tentative.

(2) The procedures presented by the referee for determining available bases in calcareous soils were adopted as tentative.

(3) The method presented by the associate referee for the determination of boron was adopted as tentative.

(4) The method submitted by the associate referee for the determination of fluorine was adopted as tentative.

(5) The method presented by the associate referee for *p*H determination of alkaline soils was adopted as tentative.

(6) The glass electrode was adopted as tentative for use in the determination of the *p*H value of soils of the humid regions.

(7) The editorial changes in the methods on soils submitted by the referee were accepted.

II. FERTILIZERS

(1) The following changes were made on pp. 19–36:

(a) P. 19, **7(a)**, the parenthetical explanation was revised and the direction, "Cool" was inserted after the first sentence.

(b) P. 23, **17**, the heading was changed to read "Citrate-soluble and Available Phosphoric Acid—Official," and the following sentence was added: "Subtract citrate-insoluble P_2O_5 from total to obtain available P_2O_5."

(c) P. 23, **19**, the following sentence was inserted: "The acid may be standardized by any of the official methods given in Appendix I."

(d) P. 23, **19(c)**, after **(b)**, the following was substituted: "or proceed as directed in any official method in Appendix I."

(e) P. 23, **19(e)** was changed to read "HgO of reagent grade, free from N."

(f) P. 24, **20(a)** was changed to read as follows: "Total capacity ca 550 or 800 cc," and **20(b)** to read "550 or 800 cc."

(g) P. 24, **19(i)**, directions for methyl red were changed to read as follows: "Dissolve 1 g of methyl red (dimethylaminoazobenzeneorthocarboxylic acid) in 200 cc of alcohol."

* Compiled by Marian E. Lapp, *Associate Editor*. Unless otherwise given, all references in this report are to *Methods of Analysis, A.O.A.C.*, 1935, and the methods are edited to conform to the style used in that publication.

(h) P. 29, **42(b)**, line 2, after "H_2PtCl_6" the words "and a Pt soln containing equivalent of 0.5 g of Pt (1.05 g H_2PtCl_6)" were added.

(i) P. 30, **44(a)**, line 1, after "50 cc" the words, "or some factor weight" were added.

(j) P. 36, **62**, "500 cc Wagner flask" was changed to "500 cc cylindrical shaking flask (Wagner)."

(2) The last sentence of **19(g)**, p. 23, was changed to read as follows: "A soln having a sp. gr. of 1.36 or higher may be used" (final action).

(3) The changes made last year in the official methods for the determination of P_2O_5 (*This Journal*, **22**, 70) were adopted as official (final action).

(4) Diglycol stearate was adopted as a reagent in the preparation of the potash solution and the directions, p. 29, **42**, were amended to read as follows:

(c) *Diglycol stearate soln.*—Dissolve 20 g of diglycol stearate Tech. in 1 liter of equal parts of benzol and ethyl alcohol (to prevent frothing), (official, final action under suspension of the rules).

(5) The words "and 1 cc of diglycol stearate when necessary to prevent foaming" were added after the word "soln" in line 2, p. 30, **43(a)**, (official, final action under suspension of the rules).

(6) The method for the determination of calcium oxide in stock feeds (p. 347, **44**) was adopted as a tentative method for the same determination in fertilizers and bromophenol blue was specified as indicator.

(7) Method 2, outlined by the Associate Referee on Copper, was adopted as tentative.

(8) The method for the determination of magnesia in water-soluble compounds, p. 34, **54**, as modified by the associate referee (*This Journal*, **21**, 77), was adopted as official (first action).

(9) The Bartlett-Tobey method for the determination of acid-soluble magnesia (*This Journal*, **22**, 270), as amended by the associate referee, was adopted as official (first action).

(10) The method submitted by the Associate Referee on Magnesia, labeled "Volumetric Modification," was amended as suggested and adopted as tentative for the determination of acid-soluble magnesium.

(11) The method for determining acid-soluble manganese in fertilizers and manganese salts (*This Journal*, **21**, 292), as amended by the associate referee, was adopted as official (first action).

(12) The colorimetric method for the determination of acid-soluble manganese presented by the associate referee last year (*This Journal*, **22**, 279), and again this year, was adopted as tentative.

(13) On p. 18, **4**, "Moisture," the word "not" was deleted (first word in parentheses).

(14) The beaker method for the determination of water-insoluble nitrogen (*This Journal*, **22**, 268) was adopted as official (first action).

(15) The following paragraph was added to the Devarda method (pp. 26–27, **33**), "In the analysis of nitrate salts, proceed as directed above, but use 25 cc of a nitrate soln equivalent to 0.50 g of the sample" (final action).

III. SEWAGE*

IV. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or other changes.

V. AGRICULTURAL DUST*

VI. INSECTICIDES, FUNGICIDES, AND CAUSTIC POISONS

(1) Pars. **25** and **26**, p. 48, were deleted.

(2) The following sentence was added to **3(e)**, p. 41, "Add ca 1 cc of Hg, shake, and allow the starch to stand over the Hg."

(3) The following words were deleted from **87**, p. 56, "or gravimetrically, weighing the chloride."

(4) The tentative distillation method for the determination of fluorine, **18, 19**, p. 46, was deleted.

(5) Method I, submitted by the associate referee for the determination of fluorine in water-soluble or water-insoluble insecticides in the absence of gelatinous silica, boron, and aluminum salts, was adopted as tentative.

(6) Method II, submitted by the associate referee for the determination of fluorine in water-soluble insecticides in the absence of boron, ferric, and aluminum salts, and large quantities of pyrethrin powder, was adopted as tentative.

(7) Method III, submitted by the associate referee for the determination of fluorine in insecticides was adopted as official (first action).

(8) The method submitted by the referee for the determination of pyrethrin I in pyrethrin powder (*This Journal*, **21**, 78) was adopted as official (first action).

(9) The method submitted by the referee for the determination of pyrethrin II in pyrethrin powder (*This Journal*, **22**, 575) was adopted as tentative.

(10) The method submitted in 1938 by the referee for the determination of rotenone in derris and cube powders (*This Journal*, **21**, 148) and amended by the insertion, after the words "powdered root," of the following clause, "and 10 g of decolorizing carbon," was adopted as official (first action).

(11) The method submitted by the associate referee for the determination of ether extract in derris and cube powders was adopted as official (first action).

(12) The method for the determination of phenol coefficient (p. 68) was adopted as official (final action).

* Subjects for future study.

(13) The method submitted by the associate referee for the determination of pyrethrin I in pyrethrin extracts in mineral oils (*This Journal*, **21**, 78; **22**, 575) was adopted as tentative.

VII. CAUSTIC POISONS

No additions, deletions, or other changes.

VIII. NAVAL STORES

(1) The following tentative methods (pp. 75–83, inclusive) were advanced to the status of official (first action): Sampling, **1**; Acid Number, **2**; Toluol-insoluble Material, **5**; Sampling of Rosin, **10**; Determination of Grade, **11**; Specific Gravity, **13**; Refractive Index, **14**; Distillation, **15**; and Correction for Variation in Atmospheric Pressure, **17.**

(2) The sulfuric-fuming nitric acid method for the determination of mineral oil in turpentine, **20**, was deleted.

(3) In the tentative method for acid number, **2**, p. 75, the following clause was inserted at the end of the first sentence, line 2, "(denatured, Formula No. 30; Bur. Int. Rev. Formula 30 contains 1 volume of methyl to 10 volumes of 95% alcohol)." In the third line the words, "and cool to room temp." were deleted.

(4) The first four lines of the saponification number method, **4**, p. 75, were changed to read as follows: "Weigh accurately 2 g of rosin sample into 250 cc Erlenmeyer flask, and bring into soln in 25 cc neutral 95% alcohol (Formula No. 30 is suitable). Add 20 cc of alcoholic KOH soln **(XXXI, 22)**, allowing pipet to drain for definite time. Connect flask to reflux condenser (air condenser tube, 5 mm I.D. 32″ long will suffice), bring to boil on steam bath or hot plate, and hold at that temp. exactly 1 hour. Cool to room temp. and titrate with 0.5 *N* HCl . . . ."

(5) In **6**, line 6, p. 76, the words "in a boiling water oven" were changed to read, "at 105–110°."

(6) The fuming sulfuric acid methods, **18**, p. 82, fourth from last line, after "92.38%," was changed to read as follows: (100.92% equivalent H_2SO_4). The equivalent H_2SO_4 content of this acid must not vary more than $\pm 0.15\%$ H_2SO_4 from the above figure. Keep acid in small bottles and protect against absorption of moisture from air." In **19**, line 2, "a little at a time" was inserted after the word "pipet," and in line 10, "conc" was inserted before "H_2SO."

IX. PAINTS, PAINT MATERIALS, AND VARNISHES

No additions, deletions, or other changes.

X. LEATHERS

No additions, deletions, or other changes.

XI. TANNING MATERIALS

No additions, deletions, or other changes.

XII. PLANTS

(1) The directions for the standardization of the potassium permanganate reagent used in the tentative volumetric permanganate method for the determination of reduced copper (**47**, p. 135) was changed to conform with the directions given in Appendix I.

(2) The tentative method for determining reduced copper by electrolytic deposition from sulfuric and nitric acid solutions (**48(c)**, p. 136) was deleted.

(3) Sections **49(a)** and **(b)**, p. 136, were changed as recommended by the associate referee.

(4) The tentative method for the determination of starch by diastase, **50**, p. 136, was deleted.

(5) The following methods were made official (final action):

(1) Directions for Sampling (**1**, p. 121).
(2) Iron and Aluminum (**6**, p. 122).
(3) Method for Iron Only (**7**, p. 122).
(4) Chlorine (**38**, p. 131).

(6) In the official method for the determination of magnesium (**12**, p. 124) "5 cc" in the 6th line was changed to "25 cc."

XIII. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

(1) The following changes were made:

P. 144, **5(b)**, line 4.—After words "add 3 cc of 1 *N* H_2SO_4" the phrase "(except in determining malic acid)" was inserted.

6.—"**XXVI, 26**" was changed to "**XXVI, 27 or 29.**"

8.—"**XXVI, 32**" was changed to "**XXVI, 34.**"

P. 145, **14** was changed to read, "Use the value for reducing sugars before inversion, **13.**"

16 was changed to read, "Proceed as directed under **XXVII, 8**, using quantity of sample that contains not more than 10 g of solids."

17 was changed to read, "Using ash obtained under **16**, proceed as directed under **XXXIV, 12.**"

18 was changed to read, "Using soluble ash obtained under **17**, proceed as directed under **XXXIV, 13.**"

19 was changed to read, "Using insoluble ash obtained under **17**, proceed as directed under **XXXIV, 14.**"

21.—Heading was changed to read, "Preservatives and Artificial Sweeteners."

P. 146, **29**, line 3.—"**23**" was changed to "**26.**"

P. 147, **30**, line 2.—"Add 22 cc of alcohol . . . of H_2O" was changed to read, "add 32 cc of alcohol and in the case of sirups or flavors ca 300 cc of H_2O."

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

(1) The following changes were made in the methods for beer:

P. 148, **1,** line 4.—Temperature of 20–25° was specified.

2.—The direction, "Filter beers showing opacity," was added.

4.—The clause, "and report to second decimal place," was added.

P. 149, **7.**—Methods (a) and (c), Real Extract, were adopted as official (final action).

8.—To line one the clause, "and report to the first decimal place," was added.

P. 150, **9.**—"And report to the first decimal place," was added.

10.—"Express results to nearest 0.01%," was added.

11.—"1 cc of 0.1 *N* alkali = 0.006 g of acetic acid" was added.

P. 151, **19, 20.**—The method for the determination of carbon dioxide was deleted.

P. 152, **21.**—The method for sulfur dioxide was deleted, and the method described by the associate referee was adopted as official (first action).

22.—The last sentence "Abnormal beer gives etc." was deleted.

26.—The tentative method for pasteurization was deleted.

P. 158, **46(b).**—Reagent was changed to following: "*Fehling's soln.*—Standardize as directed in **XXXIV, 32, 33.** Check soln from time to time by estimating its oxidizing value against a standard soln of invert sugar according to customary analytical procedure."

P. 161, **51,** line 2, and **55,** line 9.—"Gypsum plate" was changed to read "in the cavities of a porcelain plate." The formula was changed to read:

$$\text{"Extract in flakes} = \frac{\text{Total extract—extract in 60 g of malt}}{40} \times 100.\text{"}$$

P. 162, to **56,** was added the sentence, "See **53, 54,** and **55.**"

(2) The method submitted by the associate referee for the determination of total acidity was adopted as tentative.

(3) The following method for H-ion concentration of beer was adopted as tentative: "Decarbonate sample, **1,** and determine *p*H electrometrically. Report results to nearest 0.05 unit."

(4) The method presented by the associate referee for the determination of end fermentation as increase in the degree of fermentation was adopted as tentative.

XV. WINES

(1) The modified Peynaud procedure for the determination of volatile acidity of wines (*This Journal,* **22,** 210) was adopted as tentative.

(2) Method II, for the determination of total volatile acidity, **24**, p. 167, was changed as follows: The line after the title was deleted, and the determination was changed as directed by the associate referee.

(3) The tentative method for the determination of total acidity—exclusive of SO_2, **25**, p. 167, was changed as suggested by the associate referee.

(4) The notation "(Especially adapted to wine)," (p. 441, **33**) was deleted.

(5) The method presented by the associate referee for the determination of sulfurous acid was adopted as tentative.

XVI. DISTILLED LIQUORS

(1) The method presented by the associate referee for the determination of acids was adopted as official (first action) to replace the method for acidity (p. 170, 8).

(2) The method for the determination of aldehydes presented by the associate referee was adopted as official (first action).

(3) The method for the determination of benzaldehyde in cordials (p. 183, **55, 56**) was adopted as official (first action).

(4) The method for the determination of volatile esters in cordials (p. 181, **46**) was adopted as official (first action).

(5) The qualitative test for gamma undecalactone (p. 181, **47**) was adopted as official (first action).

(6) The method for the determination of total solids from residue of the dealcoholized sample (p. 180, **37(c)**) was adopted as official (first action).

(7) The Trillat method, the Riche-Bardy method (pp. 173, 174, **19, 20**), and the modified Denigè's method (p. 174, **21, 22**) were deleted.

(8) In the official method for the determination of fusel oil, the second paragraph on p. 173 was deleted and the following sentence was substituted, "It is preferable to use ground-glass connections thruout."

(9) The method submitted by the associate referee for the determination of methyl alcohol was substituted for the modified Denigès' method, **21, 22**, p. 174.

(10) The modified Marsh test (p. 179, **33**) was made official.

(11) The following changes were also made:

In **37(c)**, p. 180, "residues" was changed to read "refractive index."

In **46**, p. 181, "proceed" was changed to "steam distil."

On p. 182, **51** was changed to read, "Using ash obtained under **50**, proceed as directed under **XXXIV, 12.**"

On p. 182, **52** was changed to read, "Using the soluble ash obtained under **51**, proceed as directed under **XXXIV, 13.**"

On p. 182, **53** was changed to read, "Using the insoluble ash obtained under **51**, proceed as directed under **XXXIV, 14.**"

XVII. BAKING POWDERS AND BAKING CHEMICALS

(1) The methods presented last year by the referee (*This Journal*, **22**, 74) for the quantitative determination of total tartaric acid, cream of tartar, and free tartaric acid in tartrate baking powders were adopted as official (final action).

(2) The present official method for the determination of total tartaric acid (p. 186, **13**) was dropped (final action under suspension of the rules).

XVIII. COFFEE AND TEA

The tentative method for the determination of volatile oil in tea (p. 196, **40**) was deleted.

XIX. CACAO BEAN AND ITS PRODUCTS

(1) Par. 9, p. 198, line 2, "Add two 100 cc . . . addition" was changed to read "Add two 100 cc portions of ether, shake, centrifuge, and decant supernatant liquid after each addition."

(2) Par. **10**, p. 199, line 11, the letter "P" was inserted after the first "20°" and the letter "I" was inserted after "invert reading."

(3) Par. **12**, p. 200, 3rd line from bottom, "bell" was changed to read "bell-jar."

(4) Par. **26**, p. 204, line 4, "$NaNO_3$ soln" was changed to read "$NaNO_3$ crystals."

(5) The method for the determination of shell in cacao nibs (cocoa nibs) submitted by the referee was adopted as tentative.

XX. CEREAL FOODS

(1) The magnesium-acetate method for the determination of ash published in *This Journal*, **20**, 69, was adopted as official (final action), and substituted for the present tentative method (p. 207, **6**, **7**).

(2) The method outlined by the associate referee for the determination of H-ion concentration, specifying sulfonphthalein as indicator, was adopted as official (first action) for flour, bread, other baked products, and macaroni.

(3) The methods proposed by the associate referee for the determination of fat acidity in grain, flour, corn meal, and whole wheat flour were adopted as tentative.

(4) The modified method proposed by the associate referee for the determination of reducing and non-reducing sugar in flour was adopted as official (first action).

(5) The tentative method for the determination of sugars (p. 209, **15**) was deleted.

(6) The quick-ashing method submitted by the associate referee for the determination of chlorine in fat extracted from flour was adopted as official (first action).

(7) Procedures **34, 35, 36,** and **37,** pp. 214–15, for the determination of chlorine were deleted.

(8) As modified by the associate referee the tentative method for the determination of CO_2 in self-rising flour (p. 208, **9**) was adopted as official (first action).

(9) The method for the detection of soya flour in cereal products published in *This Journal,* **17,** 329, was adopted as tentative, and the indicator was changed from bromophenol to bromothymol blue.

(10) The method submitted by the associate referee for the determination of ash in the original flour of phosphated and self-rising flour was adopted as official (first action).

(11) The following official methods for flour were adopted as tentative for the same determinations in rye, oats, corn, buckwheat and their products: Moisture (p. 206, 2); Ash (p. 207, 5); Fat (p. 208, 10); Fiber (p. 208, 12); Protein (p. 209, 16, with factor 6.25).

(12) The following official methods for bread were adopted as tentative for the same determinations in baked products other than bread not containing fruits: Moisture (p. 222, 51, 52); Ash (p. 224, 56); Protein (p. 224, 58); Fat (p. 224, 59); Fiber (p. 224, 60).

(13) The method for the determination of apparent viscosity published in *This Journal,* **20,** 380, was adopted as official (final action).

(14) The official method for the determination of apparent viscosity acidulated flour (p. 221, **47, 48, 49**) was deleted.

(15) The following additional changes were made in the chapter on Cereal Foods:

P. 206, **2(b).**—The clause "should contain etc." was changed to read "Calcium carbide or reignited quick lime is a satisfactory drying agent."

P. 208, **11.**—The temperature "98–105°" was changed to read "100°"; the temperature "90–105°" was changed to read "100°"; and the drying period was changed from 75 minutes to read "ca 90 min."

P. 211, 212, **27.**—The temperature "98–105°" was changed to read "100°," and the sentence reading "The filtrate should be perfectly clear" 3rd line from the end of the paragraph (p. 212) was deleted.

P. 214, **33,** line 3.—The solvent was changed from "gasoline" to "50 cc petroleum benzin."

P. 223, **54,** 2nd par., line 2.—"Add 1 cc of NaOH . . . bath 1 hour" was changed to read "add 4 cc of soda glycerol soln, **XXXI, 26,** and saponify as directed under **XXXI, 27.**"

P. 224, **55.**—The following phrase was added to the last line of the paragraph: "On the assumption that whole milk was used."

P. 229, **77.**—The status of the method for lipoids and lipoid P_2O_5 was advanced to official (final action).

P. 229, **76.**—The status of the method for water-soluble protein nitrogen was changed back to tentative.

P. 229, **79.**—"Ligroin" was changed to read "petroleum ether, with boiling point below 60°."

P. 209, **15.**—The method for "Sugars—Tentative" was also placed under the sections "Bread" and "Products Other Than Bread."

P. 213, **31.**—The explanatory phrase "Applicable to uncooked cereal products" was added to the title "Starch—Tentative."

P. 224, **57.**—The status of the method for chlorides in ash was changed to official (final action).

P. 228, **69.**—The status of the method for total solids and moisture was changed to official (final action).

XXI. COLORING MATTERS IN FOODS

(1) The following change was made in method **16(b),** p. 245, under "Identification": Lines 4 and 5, last two sentences were changed to read as follows: "Dry filter, add a drop of $SnCl_2$ soln, and again dry carefully. If the color turns purple presence of annatto is confirmed."

(2) The Association was authorized to rename the colors used in foods, drugs, and cosmetics to accord with the nomenclature used in the Federal Food, Drug, and Cosmetic Act.

(3) The methods for the determination of arsenic and lead were revised to conform to these methods given under Chapter **XXIX.**

XXII. DAIRY PRODUCTS

(1) The stirrer method for preparation of butter sample presented by the associate referee was adopted as tentative.

(2) The tentative method for the determination of moisture, fat, and salt (p. 288, **79**) was deleted.

(3) The tentative method for mold mycelia in butter (*This Journal,* **22,** 76) was modified to include the alternative staining procedure recommended by the associate referee.

(4) The editorial changes suggested by the associate referee in the method for determination of fat in milk (p. 267, **19**) were accepted.

(5) The clarifying definition of the temperature of mixing (*This Journal,* **21,** 84) in the method for the determination of fat in butter (p. 289) was deleted as suggested by the associate referee.

(6) The method described by the Referee on Gums in Foods for detection of gums in soft curd cheese was adopted as tentative.

(7) The tentative method for the detection of gums in cheese (p. 295, **106**; *This Journal,* **18,** 79) was dropped.

XXIII. EGGS AND EGG PRODUCTS

(1) The tentative method for the determination of unsaponifiable matter (p. 300, **13**) was deleted.

(2) The official method for the determination of chlorine (p. 301, **16**)

was amended as recommended by the associate referee (*This Journal*, **22**, 77) and made official (final action).

(3) The method for the determination of dextrose and sucrose (p. 301, **18**), as amended by the associate referee (*This Journal*, **22**, 77), was adopted as official (final action).

XXIV. FISH AND OTHER MARINE PRODUCTS

The tentative methods for the determination of ash, salt, and total nitrogen (*This Journal*, **21**, 86) were adopted as official (first action).

XXV. FLAVORING EXTRACTS

(1) The procedure presented by the referee for the quantitative precipitation of β-ionone as *m*-nitrobenzhydrazide (*This Journal*, **22**, 383) was adopted as official (first action), and it is to be published under the heading, "Flavoring Extracts: β-Ionone, Quantitative (Applicable to pure solutions of not over 100 mg of β-ionone in 5 cc of alcohol)."

(2) The procedure for β-ionone in raspberry flavor published last year (*This Journal*, **22**, 386) was adopted as official (first action) for beverage concentrates, with the precaution suggested by the referee this year added.

XXVI. FRUITS AND FRUIT PRODUCTS

(1) The changes recommended by the referee last year (*This Journal*, **22**, 78) in the methods for the analysis of preserves and jams were adopted as official (final action).

(2) The colorimetric method presented by the associate referee for the determination of P_2O_5 in fruits and fruit products was adopted as tentative.

(3) The following changes suggested by the associate referee in the methods for the determination of ash (p. 321, **9**) were accepted (final action, under suspension of the rules):

Total Ash, **9**, 2nd par., was changed to read as follows: "If the ash of the water-soluble portion only is desired, evaporate on steam bath to dryness 100 cc of prepared soln 2(b_1) or 2(c_1) and proceed as directed under **XXVII, 8.**

XXVII. GRAIN AND STOCK FEEDS

(1) The method presented by the Associate Referee on Ash was substituted for the present official method (p. 336, **8**) and made official (first action).

(2) The method for the determination of calcium oxide in mineral feeds (p. 347, **44**) was adopted as official (first action).

(3) The qualitative tests for proteins (p. 337) were made official (final action).

(4) The modified Roese-Gottlieb method for the determination of fat in dried milk products (p. 339, **24**) was made official (first action).

(5) The method for the determination of water-soluble acidity (p. 346, **38**) was made official (first action).

(6) The methods for the determination of grit in poultry and similar feeds and for bone in meat scrap or tankage (p. 347, **42, 43**) were made official (first action).

(7) The methods for the determination of ferrous sulfate, copper sulfate, and potassium iodide (**49, 50, 51,** p. 349) were adopted as official (first action).

(8) The tentative method submitted and amended by the associate referee for the determination of manganese (*This Journal,* **21,** 292; **22,** 279), was adopted as official (first action).

(9) The method presented by the associate referee for detecting starch in condensed or dried milk products was adopted as tentative.

XXVIII. MEAT AND MEAT PRODUCTS

(1) The title of the tentative method for added water in sausage and similar products was changed to read, "Added Water in Sausage."

(2) As recommended by the referee, the tentative method for the determination of gelatin (p. 367, **62**) was modified to make it conform to the method for meat (p. 354, **8**).

XXIX. METALS IN FOODS

(1) The tentative method for the determination of lead (pp. 381, **16(a)**; 387, **25(a)**; and 388, **26(a)**) was modified as suggested by the referee.

(2) The editorial changes suggested by the referee for the official rapid method for the determination of lead (pp. 391–3, **31,** and **32**) were accepted.

(3) The tentative method for the determination of mercury (pp. 393–5, **24, 25,** and **26**) was changed as recommended by the associate referee.

(4) The method presented by the associate referee for the determination of zinc was adopted as tentative.

XXX. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

XXXI. OILS, FATS, AND WAXES

(1) The following precautionary statement was inserted in the method for determining cholesterol and phytosterol (p. 418, **33**): "Not applicable in presence of hydrogenated soybean oil."

(2) The methods for sampling and analyzing cottonseed (p. 423) were changed as follows: Sections **47–49,** were replaced by subparagraphs **(a)** to **(e),** inclusive, of Section **3,** Section **4** in its entirety, and subparagraph **(a)** of Section **5,** up to and including the word "container," of "Methods of Drawing and Preparing Official Samples of Cottonseed," approved July 5, 1939, by the Chief of the Agricultural Marketing Service, U. S. Department of Agriculture; Sections **50–58** were replaced by Section **1,**

(b) with the first sentence changed to read as follows: "Separate foreign matter by passing sample over a 6-mesh screen and picking out other particles of foreign matter not so removed," and by Sections **2–7**, inclusive, of "Methods of Chemical Analysis and Grade Calculations for Cottonseed," approved July 5, 1939, by the Chief of the Agricultural Marketing Service, U. S. Department of Agriculture. Since these substitutions merely involve changes in methods in official use by licensed cottonseed graders of the U. S. Department of Agriculture, the amended methods were made official (final action).

(3) The omission of the caption "Official" in the method mentioned in the previous recommendation was rectified.

(4) The opening sentence of subparagraph **(a)** of Section **3** of the sampling methods mentioned in (2) were changed editorially by the deletion of the word "licensed" and by the substitution of the word "suitable" for "approved" here and also in the fifth and seventh lines of Section **1, (b)** of the same analytical methods.

XXXII. PRESERVATIVES AND ARTIFICIAL SWEETENERS

No additions, deletions, or other changes.

XXXIII. SPICES AND OTHER CONDIMENTS

(1) The tentative method for the determination of volatile oil in spices (p. 444, **16**) was made official (final action) for marjoram and sage.

(2) In the method for the determination of acid-insoluble ash in spices (p. 445, **5**) the phrase "until carbon-free" was added after the word "ignite" (line 3).

(3) The tentative method for the determination of reducing sugars before inversion (Salad Dressings, p. 454, **45**) was amended by the referee as follows:

In **45**, par. 2, line 2 etc. was changed to read "In such instances, remove the oil according to the principle of the Roese-Gottlieb method, **XXII, 19**, using 1 cc of NH_4OH and 5 cc of alcohol for each gram of sample; transfer residue to 250 cc flash with alcohol, 50% by volume, and proceed as directed under **XXVII, 28**, and **XXXIV, 37** and **38**."

(4) The tentative method for the determination of reducing sugars after inversion (Salad Dressings, p. 454, **46**) was amended as suggested by the referee as follows:

In **46**, "XXXIV, **23(b)**" was changed to read "XXXIV, **23(b)** or **(c)**" and "**XXXIV, 37**" was changed to "**XXXIV, 37** and **38**."

(5) In the heading of the tentative method for the determination of iodine number of paprika (p. 450, **26**) the words "Qualitative test for presence of foreign oil," were substituted for the words, "Indication of presence of olive oil—Qualitative test," and the method was made official (first action).

(6) The method submitted by the Referee on Gums in Foods for the

determination of gums in mayonnaise and French dressings (*This Journal*, **22**, 607) was adopted as tentative.

XXXIV. SUGARS AND SUGAR PRODUCTS

(1) The method for the determination of sucrose and raffinose by polarization before and after treatment with two enzymes (p. 474, **24**, and **25**) was made official (final action).

(2) Somogyi's modification of the Shaffer-Hartman microchemical method for reducing sugars was adopted as tentative.

(3) The methods for "Ash—Official" (p. 465, **8, 9, 10**), were revised as directed by the associate referee.

(4) The method presented by the refere for electrolytic deposition from nitric acid solution was adopted as tentative and replaces the present method, **42**, p. 481.

(5) A new column was inserted in the Munson-Walker reducing sugar table to provide for the copper equivalents of pure levulose.

(6) New equivalents of pure invert sugar were presented by the associate referee as substitutes for those given in the Munson-Walker table.

(7) The conversion factors of the different saccharimeter scales were revised as suggested by the referee.

(8) The volumetric thiosulfate method (**39**, p. 480) and the volumetric permanganate method (**41**, 480) were revised as suggested by the referee.

(9) Tables **6** and **7, XLII**, were revised as suggested by the Associate Referee on Drying, Densimetric, and Refractometric Methods.

(10) In Methods **3** and **4**, pp. 462, 463, the pressure of "100 mm of Hg" was changed to read "50 mm of Hg."

XXXV. VEGETABLES AND VEGETABLE PRODUCTS

(1) The method submitted by the associate referee for the determination of chlorides in tomato products (rapid method) was adopted as tentative.

(2) The first sentence of the method for the determination of total solids in tomato products (p. 499, **16**) was amended to read as follows: "Weigh into a flat-bottomed dish a portion of sample of such size that the dry residue will not be less than 9 mg nor more than 12 mg per sq. cm. of drying surface."

(3) The following sentence was added after the heading of the method for physical examination of vegetables and vegetable products (p. 497, **1**), "Applicable to canned products only."

(4) In **3**, p. 497, "XXVII, 5" was changed to "XXVII, 2."

(5) To **2**, first paragraph, p. 497, was added the following, "Without shifting the product, so incline the sieve as to facilitate drainage of the liquid. With the exception of very soft products, such as tomatoes, carefully invert by hand all pieces containing cups or cavities if they fall

on the sieve with cups or cavities up. Cups or cavities in soft products may be drained by tilting the sieve, but no other handling of these products while draining is permissible"; the first sentence of par. 2 of the same section was changed to read: "Allow the material on the sieve to drain 2 min. . . . "; the method was given the explanatory caption, "Applicable to canned products only"; and the method was incorporated by reference under a similar caption under Fruits and Fruit Products (p. 319, 2).

(6) The method for yeasts and spores (29, p. 501, par. 2) was amended by the placing of a period after "cover-glass," line 4, and by changing the next sentence to read as follows: "Discard any amount showing uneven distribution, absence of Newton's rings, or liquid that has been drawn across the moat and under the cover-glass."

XXXVI. VITAMINS

The method for vitamin D milk (*This Journal*, 21, 90) was revised as suggested by the associate referee.

XXXVII. WATERS, BRINE, AND SALT

(1) The thorium nitrate filtration method for the determination of fluorine recommended by the referee was adopted as tentative.

(2) The tentative method for the determination of fluorides (p. 508, 22–26, inclusive) was dropped.

XXXVIII. RADIOACTIVITY

The tentative method and the diagram for radioactivity determination were revised to conform to modern practice.

XXXIX. DRUGS

(1) The tests for physostigmine and stovaine proposed by the associate referee were adopted as tentative.

(2) The status of the microchemical tests for the alkaloids enumerated by Subcommittee B were advanced from official (first action) to official (final action), (see p. 55).

(3) The status of the microchemical methods for the synthetics named by the associate referee was advanced from official (first action) to official (final action), (see p. 56).

(4) Method I submitted by the associate referee for the separation of acetophenetidin, acetylsalicylic acid, and salol was adopted as tentative.

(5) The method submitted by the associate referee for the determination of elixir of terpin hydrate and codeine was adopted as tentative.

(6) The sublimation method presented by the associate referee for the determination of nicotinic acid in tablets was adopted as tentative.

(7) The method for the determination of ephedrine in inhalants (p. 557,

42) was amended by the deletion in the next to last line of the phrase, "on a steam bath with moderate heat."

(8) The method for the determination of caffeine (p. 545, **7**, line 1) was amended by the addition, after the word "add" of the following words: "1 cc of 9 *N* H_2SO_4 and."

(9) The method presented by Matchett and Levine for the determination of procaine, which is not affected by the presence of heroine, codeine, or cocaine, was adopted as tentative.

(10) The status of the methods recommended by the Referee on Drugs was advanced from official (first action) to official (final action), (see p. 59).

(11) The methods for the determination of ipomea and jalap (pp. 566–**7, 72, 73**) were deleted as assays are now given in the National Formulary.

(12) The method for determining the swelling factor of psyllium (p. 587, **126**) was deleted as it can now be found in the National Formulary.

(13) The status of the methods recommended by the Referee on Drugs was advanced from tentative to official (first action), (see p. 59).

(14) The method for the determination of iodoform in ointment (p. 594, **149**) was adopted as official (final action), under suspension of the rules.

(15) Both methods for the determination of santonin (pp. 587–8, **128, 129**) were adopted as official (final action). They will be presented under one heading, "Santonin in Mixtures."

(16) The reagent in the official quantitative method for the determination of pyramidon (p. 573, **87**) for holding aminopyrine in solutions was changed from hydrochloric acid to sulfuric acid (final action under suspension of the rules).

(17) The official method for the determination of camphor (p. 560, **51**) was amended (final action) by the insertion of the explanatory sentence, "Not applicable to synthetic camphor."

(18) The official method for the determination of barbital and phenobarbital (p. 582, **112**) was amended (final action) by the addition at the end of the paragraph of the following sentence: "Determine the m.p. to check purity of residue."

XL. MICROBIOLOGICAL METHODS

(1) The methods for frozen egg products published in *This Journal*, **22**, 625, as revised by the associate referee, were adopted as tentative.

(2) The methods presented by the Associate Referee on Canned Vegetables were adopted as tentative.

(3) The methods for detecting and estimating thermophilic bacteria in sugar recommended by the associate referee were adopted as tentative.

XLI. MICROCHEMICAL METHODS

No additions, deletions, or other changes.

APPENDIX I. STANDARD SOLUTIONS

(1) As amended, the method for standardization of acid solutions with borax recommended by the associate referee last year (*This Journal*, **22**, 102), was adopted as official (final action).

(2) The method submitted by the associate referee for standardization of acid solutions with sodium carbonate (*This Journal*, **22**, 103) was adopted as official (final action).

(3) The method for standardization of sodium hydroxide solutions (p. 681) was adopted as official (final action).

(4) The method proposed by the associate referee for preparation and standardization of sulfuric acid solutions was adopted as tentative.

(5) The method proposed by the associate referee for standardization of potassium permanganate solutions was adopted as official (first action).

No report was given by the Committee on Standard Scale for Immersion Refractometer.

REPORT OF COMMITTEE TO CONFER WITH AMERICAN PUBLIC HEALTH ASSOCIATION ON STANDARD METHODS OF MILK ANALYSIS

By E. M. Bailey, *Chairman*

This Committee collaborated with the Editorial Committee of our Association during the past year in preparing the chemical section of the 7th edition of "Standard Methods for the Examination of Dairy Products." The collaboration consisted of introducing into their publication, methods from our *Methods of Analysis* so far as they related to dairy products. The collaborative work has been completed and the book has been issued, with due credit given to the A.O.A.C.

Approved.

REPORT OF THE A.O.A.C. REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE

In the report presented one year ago we emphasized the importance of chemistry in a plant protection research program. Many of the major projects in progress today are aiming to discover organic insecticides and fungicides that are harmless to man and thus overcome the objections to the mineral substances largely relied upon in former years. Much attention is also being given to developing prevention through the better feeding and nutrition for crops as well as lessening the cost of insect and disease control. Research on all of these objectives receives much help from the chemist.

The crop Protection Institute is conducting at present research projects for the following organizations:

(1) *Carbide and Carbon Chemicals Corporation*—
  (a) Exploration of possible fungicides.
  (b) Exploration of possible insecticides.
(2) *Sherwin-Williams Company*—
  (a) Field studies of the fungicidal activities of basic copper arsenate on potatoes.
(3) *United States Rubber Company*—
  (a) Fruit fungicides.
  (b) Seed protectants.
  (c) Insecticide studies.
    (1) Contact insecticides.
    (2) Organic repellents and stomach poisons.
    (3) Moth-proofing materials.
    (4) Stabilization of derris.
(4) *Standard Chemical Products, Inc.*—
  (a) Utilization of Sinox as a herbicide.
  (b) Development of summer spray formulas with the dormant spray Elgetol.
(5) *General Chemical Company*—
  (a) Various copper sprays on small fruits and vegetables.
  (b) The use of adhesives with arsenates in order to reduce the number of applications.
(6) *Dow Chemical Company*—
  (a) Studies of dinitro compounds.
(7) *Rhom and Haas Company*—
  (a) Studies with cuprous oxide.
(8) *Shell Corporation*—
  (a) Insect repellents.
(9) A Survey of soil-less culture.

These projects are being conducted in cooperation with the following institutions:

Boyce Thompson Institute
Indiana Experiment Station
Maine (Aroostook) Sub-station
Connecticut (New Haven) Station
New Hampshire Station
Maryland Experiment Station
California Experiment Station
Louisiana Experiment Station
North Carolina Experiment Station
Geneva, New York, Experiment Station

We recommend that whenever the opportunity offers the chemists of this Association cooperate with the entomologists, plant pathologists, and plant physiologists and aim to make the research in these fields more thorough.

H. J. PATTERSON
W. H. MACINTIRE

Approved.

REPORT OF SECRETARY-TREASURER

By W. W. SKINNER

During the past year, unfortunately, there has been an unusual number of deaths of members. The list of record is as follows:

Dr. Willard Dell Bigelow
Dr. William John Gascoyne
Dr. Burt Laws Hartwell
Dr. Thomas Joseph Bryan
Dr. William Marshall Allen
Dr. David Augustus Coleman
Dr. Jacob Goodale Lipman
Dr. Joseph Bridgeo Lindsey

Dr. Browne, necrologist, will present his report on each of these former members.

One resignation was received during the year, that of R. E. Lothrop, Associate Referee on Honey. Mr. Lothrop is now connected with one of the Regional Laboratories.

The attendance for 1939 has again broken the record, as the registration shows a total of 550.

As a result of a report made by the committee composed of Dr. MacIntire, Dr. White, and Dr. Kraybill, there was established by the Association, the Wiley Award of the A.O.A.C. for the preparation of papers by undergraduate students in the subject of agricultural chemistry. A circular was issued by the office of the Secretary in regard to these awards, and this circular was sent to all institutions presumably interested in the subject, and it was published in some of the scientific journals. While it was late in the year there was a fairly active response to the announcement. It was intended that any institution having a course in agricultural chemistry might submit one paper representing the product of that institution. That has been done in a number of cases. I am not able to tell you how many papers were submitted because we received in each case t he one paper selected by the faculty as being worthy of consideration by the Committee to determine who should receive the prizes: 1st prize, $300; 2nd, $200; and 3rd, $100. Papers were received in the office of the secretary and submitted to a committee of review composed of the original committee and of Dr. Bailey and Dr. Byers as additional members. This committee made a report to the Executive Committee. It will be presented as a separate report following the financial statement. The checks were authorized by the Executive Committee to be paid by the Treasurer to the persons named as winners of the awards by the Committee on Awards. It is intended, of course, that a similar award will be offered for papers to be submitted in the ensuing year, and from what we have learned we believe this effort on the part of the Association to stimulate an interest in the methods in use by the agricultural chemists is a very worthwhile undertaking.

Before reporting as Treasurer, I wish to make the usual statement abo the accounts. The affairs of the Association, a corporation, are conduct in accordance with its by-laws and the practice adopted in annual sessi some years ago. The books were audited as directed by the Associati by a public accountant (John Bisselle of Washington) and his report is follows:

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE YEAR ENDED SEPTEMBER 30, 1939

BALANCE, OCTOBER 1, 1938			
Lincoln National Bank		$ 2,764.55	
Montgomery Building and Loan Association		$ 85.54	$ 2,850.(
RECEIPTS			
Sales:			
Methods of Analysis		$ 5,296.72	
Journals		4,983.90	
Wiley's Principles		110.50	
Reprints		158.32	
		$10,549.44	
Less: Discounts allowed	$1,314.66		
Refunds	7.75	1,322.41	
Net sales			9,227.(
Other income:			
Interest on investments		$ 437.60	
Advertisements		488.80	
Total other income			926.4
Miscellaneous receipts:			
Federal-American Bank, liquidating dividend		$ 7.39	
Commercial National Bank, liquidating dividend		24.08	
H.O.L.C. bond redeemed		1,000.00	
Returned checks made good		1,143.40	
Books ordered through Association		1,281.98	
Total miscellaneous receipts			3,456.8
			$16,460.3
DISBURSEMENTS			
Expenses:			
Salaries		$ 1,327.50	
Postage		250.00	
Meeting and Association expense		398.84	
Stationery and supplies		10.50	
Auditing		150.00	
Premiums, employees' bonds		10.00	
Printing and binding		5,704.94	
Safe deposit rental		3.30	
Exchange		1.93	

Freight	$ 84.47	
Insurance on stock	5.07	
Traveling expense	300.00	
Over and short	3.45	
Storage	21.00	
		8,271.00
Miscellaneous disbursements:		
Books ordered through Association	$ 1,197.98	
Purchase of U. S. Savings Bonds	1,875.00	
Returned checks	1,267.30	
Total miscellaneous disbursements		4,340.28
BALANCE, SEPTEMBER 30, 1939		
Lincoln National Bank	$ 2,759.70	
Montgomery Building and Loan Association	1,089.39	3,849.09
		$16,460.37

BALANCE SHEET AS AT SEPTEMBER 30, 1939

ASSETS

Current Assets:			
Cash in banks:			
Lincoln National Bank	$2,759.70		
Montgomery Building and Loan Association	1,089.39	$ 3,849.09	
Accounts receivable	$1,379.17		
Less: Reserve for doubtful accounts	120.80	1,258.37	
Inventories		4,856.58	
Total Current Assets			$ 9,964.04
Investments			19,055.00
Cash in Closed Banks:			
Federal-American Bank and Trust Company		$ 18.46	
Commercial National Bank		72.26	90.72
Furniture and Fixtures			97.26
Total Assets			$29,207.02

SURPLUS

Balance, October 1, 1938	$29,374.56
Add: Net profit for year	1,070.45
	$30,445.01
Less: Adjustment to write off accounts receivable for *Methods of Analysis*, charged off in prior years	1,237.99
	$29,207.02

Approved.

W. H. MacIntire: I think Dr. Skinner covered the subject of awards thoroughly. He may not have given emphasis to one of the two objectives, and he modestly refrained from saying it was due almost entirely to his splendid management of the finances of the Association that we were in a position to launch this fine project, offering as it does such an effective memorial to Dr. Wiley, one which we feel would have been more pleasing to him than any that could be proposed. We also faced the fact that in a great many institutions there is no chair of Agricultural Chemistry, and I think the consensus is that those going out, even of the State institutions, today, frequently do not have the background that would direct their interest toward fields of chemistry. They not only do not have the background, but frequently they lack inspiration. As you know, the Regional Laboratories will employ some 800 chemists, and it seems that those men who come in should have an appreciation of chemistry and its relation to agriculture. We find it difficult to get graduates who really have a sympathetic interest in agriculture, and we felt that this award should serve as a stimulation of such interest. The fact is that chemistry has a most extensive application to agriculture, and it is very desirable that students going into the different laboratories where agriculture is the background should be better qualified.

The formal report of the Committee on Awards follows:

REPORT OF COMMITTEE ON HARVEY W. WILEY MEMORIAL AWARDS

The undersigned Committee has accorded the following rating to the papers received from the several faculties of the several departments of chemistry in accredited colleges and universities in behalf of contestants for the Harvey W. Wiley Memorial Awards.

First: "Purification of Cellulose with Acetic Acid," Harold Forest Snow, B.S., through Department of Chemistry, Massachusetts Institute of Technology.

Second: "The Utilization of Carotene from Different Oils by the Rat," Ralph D. Maby, B.S., through Department of Agricultural Chemistry, Purdue University.

Third: "The Use, Effect, and Determination of Arsenic in Florida Grapefruit and Oranges," John D. Servis, B.S., through Department of Agricultural Chemistry, University of Florida.

It is recommended that the awards of $300, $200, and $100 go, respectively, to Messrs. Snow, Maby, and Servis.

W. H. MacIntire, Chairman
E. M. Bailey
W. B. White
H. R. Kraybill
H. G. Byers

Approved.

COMMITTEE ON MOISTURE

It was moved, seconded, and carried that the Committee on Moisture be discontinued.

COMMITTEE ON STANDARDIZATION OF GLASSWARE

A motion was made that a special committee of three be appointed to study the question of the standardization of chemical glassware and to report as to policy to this Association at the next meeting. This motion was seconded and carried. The President named the following committee: G. S. Fraps, H. C. Lythgoe, and W. F. Hand.

REPORT OF AUDITING COMMITTEE

The public accountant's audit of the books of the Association of Official Agricultural Chemists, Inc., for the year ended September 30, 1939, was examined by the Committee and found to be correct. Verification was also made of the bonds on deposit.

WM. F. REINDOLLAR
DAN DAHLE

Approved.

No report was given by the Committee to Cooperate with Other Committees on Food Definitions.

REPORT OF THE COMMITTEE ON NECROLOGY

This year's Necrology Report of the Association is a long one, irreparable losses having been suffered in the passing of eight prominent former members. The first of these to be mentioned is Willard Dell Bigelow, who was President of the Association in 1909 and Editor and Secretary-Treasurer in 1912. His long services as head of the Food Division and Assistant Chief of the Bureau of Chemistry and as Director of the Research Laboratory of the National Canners' Association are too well known to require a detailed account. For comprehensive knowledge of the regulatory control of foods, of food research, and of food technology in all their numerous relationships Dr. Bigelow ranked among the foremost chemists of our time. He was prodigiously active in the work of this Association in the twenty year period between 1893 and 1913, and since that time up to his last illness had been a constant attendant at our meetings. A very appreciative sketch of his career and lovable personality is given in the obituary by Dr. F. C. Blanck in the current November issue of *The Journal.*

Another long familiar figure at our meetings whose death we have to record is William John Gascoyne, one of the Charter members of the Association, of whom now only Dr. Charles W. Dabney remains. Dr.

Gascoyne was the last survivor of the members of the three committees on nitrogen, phosphoric acid, and potash appointed at the first meeting. He was elected Vice-President of the Association in 1887, and would have succeeded to the office of President except for his withdrawal from official work to go into private business. Although not an active member since this time, he continued to maintain a deep interest in the work of the Association for over half a century and was present with us at the last, 1938, convention. A fuller account of his interesting career is given in the obituary sketch by H. B. McDonnell in the last August number of *The Journal.*

A third loss to be recorded for the past year is Burt Laws Hartwell who was a frequent attendant at our meetings in the twenty year period between 1898 and 1918. He was Referee on Soils and Ash at our 15th meeting and Chairman of Committee A on Recommendations at the 20th meeting. He presented numerous papers on the analysis of soils and fertilizers, pot experiments, lime requirements, and other subjects and was known internationally for his work on acid soils. Born at Littleton, Massachusetts, on December 18, 1865, Dr. Hartwell graduated from Massachusetts College in 1889 and obtained his Ph.D. degree from the University of Pennsylvania in 1903. He occupied positions as chemist and agronomist at the Rhode Island Agricultural Experiment Station, of which he was director from 1913 until his resignation in 1928. From then until his death on July 12, 1939, he was a writer on agricultural subjects. Although not an attendant at our meetings during the past twenty years, he contributed occasional papers, and between the 38th and 43rd meetings, as Chairman of the Crop Protection Institute of the National Research Council, assisted in presenting reports upon its work to the Association. A fuller account of his career by Homer J. Wheeler is presented in this number of *The Journal.*

Another Agricultural Experiment Station Director and former member of our Association who passed away since our previous meeting is Jacob Goodale Lipman. He was born in Fredrichstadt, Russia, November 18, 1874, and came to the United States as a boy of 14. In 1898 he obtained his B.S. degree from Rutgers University, and in 1903 his Ph.D. degree from Cornell. Becoming Assistant Chemist of the New Jersey Experiment Station in 1898, he rose rapidly through the positions of Chemist and Bacteriologist to that of Director, which he held from 1911 until his death on April 19, 1939. His first attendance at our meetings was in 1903 when he presented a paper on the "Fixation of Atmospheric Nitrogen by Bacteria." At the 1908 meeting he reported as Associate Referee on "The Determination of Calcium Carbonate in Soils" and also presented a paper on "Methods Relating to the Role of Decomposition of Organic Matter in Soil." In 1911 as Referee on Soils he read a report on "Bacterial Methods for Estimation of Soil Acidity." Dr. Lipman's last appearance

before our membership was at the 1925 meeting when he issued an announcement of the meeting of the International Congress of Soil Science which was to be held under his presidency in Washington in June 1927. He was known internationally for his work in soil science and was the recipient of many honors. A fuller account of his career and great services to agriculture is published in the Experiment Station Record for July 1939.

The fifth death among our membership to be recorded is that of Thomas Joseph Bryan, who between 1906 and 1913 took a prominent part in its meetings, serving as Referee on Fats and Oils in 1909 and 1910, and as collaborator on miscellaneous methods for analyzing baking powders from 1914 to 1928. Dr. Bryan was born at Warwickshire, England, on September 26, 1869, and coming to the United States as a child graduated from Colgate University, which later in 1914 conferred upon him the degree of Doctor of Science. He took post graduate studies in Germany at the universities of Göttingen, Heidelberg, and Freiburg, obtaining his Ph.D. degree from Freiburg in 1901. After teaching chemistry for a number of years he served as State Analyst of Illinois from 1906 to 1913. The remainder of his professional career was spent with the Calumet Baking Powder Company, of which he was Chief Chemist from 1913 to 1923, Vice-President in charge of Manufacture from 1913 to 1929, and Technical Director of the Calumet Division of General Foods Corporation from 1929 until his retirement on January 1, 1936. He was Chairman of the Division of Agricultural and Food Chemistry of the American Chemical Society in 1917, Secretary of this Division in 1919, and a member of the State Food Standards Commission of Illinois from 1924 to 1930. Although no longer active in the work of the Association during the past twenty-six years, he was a frequent attendant at our meetings. He was a prominent figure in the circles of Food Chemistry and Technology, and his passing on January 23, 1939 came as a shock to a host of friends.

A sixth member of our Association who has recently passed away, and a frequent attendant of its meetings from 1904 to 1935, is William Marshall Allen. He was born in Anson County, North Carolina, January 22, 1866, and obtained his chemical training at the universities of North Carolina and Johns Hopkins. Becoming connected with the North Carolina Department of Agriculture, as Assistant Chemist, in 1894, he remained with that Department until his retirement January 15, 1937. It was through his efforts that the State Food and Drug Law was passed in 1909. A Food Division of the Department of Agriculture was then created and he was placed in charge of the food control work. He was State Chemist from 1919 until 1932, and from that date until his retirement was Chief of the Food and Oil Division. He was President of the North Carolina Section of the American Chemical Society in 1910, Secretary of the National Association of Food and Drug Officials from 1909 until 1915, and President of the latter organization in 1930. Although not taking an ac-

tive part in our Association, he made many friends among its members. His passing on January 19, 1939 is greatly lamented.

A seventh death among our former membership, which occurred only last Friday, is that of Joseph Bridgeo Lindsey, associated for over half a century with the Massachusetts College at Amherst. Born at Marblehead, Massachusetts, on December 26, 1862, he obtained his B.S. degree from Massachusetts College in 1883 and his Ph.D. degree from Göttingen University in 1891. He was appointed Assistant Chemist at the Massachusetts Experiment Station in 1883, Associate Chemist in 1892, and Chemist in 1907, and was Vice Director from 1911 until his retirement in 1932. He was also until his retirement Goessmann Professor of Agricultural Chemistry at the Massachusetts College. Although an attendant of our meetings only between 1893 and 1897, he presented during this period several papers of importance. In 1896 he read a paper on the phloroglucin method for determining pentosans. He was Referee on Cattle Feeds at the 1896 and 1897 meetings, and in his reports suggested valuable improvements for the determination of starch, pentosans, and galactan. A long period of ill health, due to a nervous breakdown, prevented him from further participation in the work of our Association. In the field of Agricultural chemical research Dr. Lindsey made valuable contributions to the subjects of animal nutrition and dairying.

By a regrettable oversight in last year's necrology no mention was made of the death on February 25, 1938, of David Augustus Coleman, for many years head of the milling, baking, and chemical laboratory of the Grain Division of the Bureau of Agricultural Economics. His prominence as an agricultural chemist justifies the insertion of this belated notice. Born at Natick, Massachusetts, on March 15, 1892, Dr. Coleman obtained his B.S. degree from Massachusetts College in 1914 and his Ph.D. degree from Rutgers University in 1917. As Associate Referee on Cereal Products he presented reports at the 42nd, 43rd, 45th, and 47th meetings of our Association on the ash, gasoline color value, and chlorine content of flour. His contributions to cereal chemistry relate to moisture testing in grains, milling and baking qualities of wheat, rapid determination of oil in flaxseed, malting of barley, and other related subjects. He was Editor-in-Chief of "Cereal Chemistry" and rendered valuable service in the administration of the United States Grain Standards Act. His passing at the early age of 46 was a most serious loss to the cereal work of our Association, of the American Association of Cereal Chemists, and of the U. S. Department of Agriculture.

These departed members have inspired us all in past years by their loyalty to the work in which our Association is engaged, and I move you, Mr. President, that we all rise as a token of respect to their memory.

C. A. BROWNE, *Chairman*
H. C. LYTHGOE

Approved.

REPORT OF NOMINATING COMMITTEE

The Committee on Nominations wishes to present the following candidates:

President: W. W. Skinner, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

Vice-President: L. B. Broughton, College Park, Maryland.

Secretary-Treasurer: H. A. Lepper, U. S. Food and Drug Administration, Washington, D. C.

Additional Members of the Executive Committee: J. W. Sale, Washington, D. C.; G. G. Frary, Vermillion, S.Dak.; J. O. Clarke, Chicago, Ill.; W. S. Frisbie, *Ex-Officio,* Washington, D. C.

H. R. KRAYBILL, *Chairman*
H. H. HANSON
C. C. MCDONNELL

A unanimous vote was cast for the officers nominated.

REPORT OF THE COMMITTEE ON RESOLUTIONS

Whereas, the 55th Annual Meeting of the A. O. A. C. has now reached its conclusion and a large attendance has listened to the numerous and valuable reports of the referees, as well as to the interesting papers presented; be it

Resolved, that to our genial and efficient President, Walter S. Frisbie, we express our sincere appreciation of his excellent presidential address and his uniformly cooperative and courteous direction of our proceedings.

Resolved that to Dr. Paul B. Dunbar we express our thanks for his Wiley Memorial Address, entitled "A New Law Brings New Problems."

Resolved that we extend our thanks to our fellow members who have presided over the sectional meetings, as well as to those who have acted as referees and associate referees.

Resolved that we extend to our Secretary, Dr. W. W. Skinner, our sincere thanks for his work in the past and express a desire that he will honor our meetings by attendance for many years in the future.

Resolved that we extend to Miss Lapp and her associate workers our thanks for their efforts to make this meeting a success.

Resolved that through our Secretary we extend our thanks to the management of the Raleigh Hotel for the many courtesies extended to us.

Approved.

CORRECTION

In the paper, "The Freezing Point of Milk," by Lincoln M. Lampert, Vol. **XXII,** No. 4, p. 768 (1939), the second line of the last paragraph on page 770 should read "a freezing-point depression of 0.544 or less"—not "0.540."

CONTRIBUTED PAPERS

SYMPOSIUM ON THE NITROGEN-FREE EXTRACT OF FOODS AND FEEDING STUFFS

The need of a reform in the Henneberg method for evaluating feeds and foodstuffs has long been recognized. This method, although introduced three-quarters of a century ago, has continued unchanged until the present time, notwithstanding the numerous improvements that have been made in methods for the analysis of plant materials. In view of the importance of the subject it was decided by the officers of the Division of Food and Agricultural Chemistry to hold a nitrogen-free extract symposium at the Boston meeting of the American Chemical Society on September 12, 1939. A program to this end was accordingly organized with the help of various collaborators, who were invited to present papers upon the so-called nitrogen-free extract of plant materials from the viewpoints of their several specialties. The symposium attracted wide attention and a general request was made that the various contributions be made available to agricultural chemists. The papers were accordingly released by the American Chemical Society to the Association of Official Agricultural Chemists for publication in the current issue of its *Journal.*

THE ORIGIN AND APPLICATION OF THE TERM NITROGEN-FREE EXTRACT IN THE VALUATION OF FEEDING STUFFS

By C. A. BROWNE, *Chairman of the Symposium* (Bureau of Agricultural Chemistry and Engineering, Department of Agriculture, Washington, D. C.)

The ingredient of foods and feeding stuffs called nitrogen-free extract has been a subject of discussion among agricultural chemists since the term was first introduced by Henneberg and Stohmann[1] nearly eighty years ago in their classic "Contributions to the establishment of a rational method for feeding ruminants." The term has been chiefly criticized for the reason that it does not represent a single constituent, but a residuum of numerous undetermined substances of variable nutritive value, the calculation of which by difference is vitiated because of the errors involved in determining the protein, fat, fiber, and ash.

In the listed tables of the composition of feeding stuffs, nitrogen-free extract appears as the most abundant constituent, the average amount being over 47 per cent for the dry matter of hay and other forage crops and over 70 per cent for the dry substance of various root crops. The importance of enlarging our knowledge of the chemical nature, nutritive value, and physiological significance of the various components of so

[1] Beiträge zur Begründung einer rationellen Fütterung der Wiederkäuer, Vol. 1 (1860).

abundant an ingredient of our crops and crop products is therefore self-evident. It is one of numerous neglected fundamental problems of agricultural chemistry that deserve greater attention.

The adoption of the term nitrogen-free extract was brought about by the efforts of Henneberg and Stohmann to improve the current methods of feeding stuff valuation. Theirs were not the first attempts to be made in this direction. Shortly after the beginning of the nineteenth century Davy[2] valued feeding stuffs according to the amount of extract that was removed by digestion with hot water. In 1809, Thaer[3] introduced his system of hay-equivalents, using meadow hay as a standard. He estimated that 90 pounds of the dry hay of clover, or vetch, or alfalfa, or sainfoin, or 200 pounds of potatoes, or 266 pounds of carrots, or 350 pounds of rutabagas with tops, or 460 pounds of beets with tops, or 525 pounds of radishes or 600 pounds of white cabbage were equal to 100 pounds of meadow hay in feeding value. These hay values were an advance over the older methods of valuation but the failure to consider the protein fraction of the feed led to discordant results. Other observers obtained different hay values and so a mass of contradictory tables began to encumber the literature. Boussingault[4] in 1844 proposed the valuation of cattle feeds upon the basis of their nitrogen content as compared with ordinary hay of 1.34 per cent nitrogen content.

It was the great service of Henneberg to break away entirely from the old hay equivalent conception and to base the valuation of feeding stuffs on the percentage of the three major nutritive constituents—proteins, fats, and carbohydrates. Crude fiber, assumed to be indigestible, was determined by a modification of the old Einhof procedure, which consisted in removing the more easily hydrolyzed cellular constituents with successive portions of boiling dilute acid and alkali. The sum of the percentages of moisture, crude fat, crude protein (N×6.25), ash, and crude fiber subtracted from 100 gave the percentage of nitrogen-free extract. The crux of the whole nitrogen-free extract problem is the nature of the substances removed by the boiling acid and boiling alkali treatment in the determination of crude fiber. Questions of terminology bothered Henneberg not a little in the outlining of his new process. He commented as follows:[5]

> The substance called wood fiber is evidently not always the same substance since the product obtained from the same feeding stuff varies with different processes of mechanical comminution. For this reason it would be desirable to give the residue obtained by the treatment with acid and alkali a less definite designation than wood fiber (i.e., cellulose). This is especially necessary since the residues obtained from different substances by exactly the same process have a very different composition (clover hay residue, for example, being quite different from that of

[2] Elements of Agricultural Chemistry. London (1813).
[3] Grundsätze der rationellen Landwirthschaft, Vol. 1, p. 263. Berlin (1809).
[4] Economie Rurale, Vol. II, pp. 438–9 (1844).
[5] *Loc. cit.*, Vol. II, pp. 49–50 (1864).

wheat straw or from that of the feces of an animal fed on clover hay, etc.) and are essentially unlike the pure wood fiber or cellulose of the chemists. We feel it necessary, therefore, to propose for the residue in question the name "crude fiber" (Rohfaser) and will continue to retain this usage until a process is found for determining the content of feeding stuffs, etc. in true wood fiber (i.e., cellulose). From similar considerations we call the mixture of nitrogen free substance (which is determined from the difference between organic dry substance and protein plus crude fiber) "Nitrogen-free Extract." Finally for the protein substance, as calculated from the nitrogen content, the term "crude protein" is proposed in those cases where differences in the digestibility of this constituent is not considered.

The present status of feeding stuff analysis is practically the same as the one thus announced provisionally by Henneberg in 1863. That he expected this status to be modified by progress in the discovery of more improved methods of analysis is indicated from the passage just quoted. Such methods have in fact been developed in these 76 years, and one of the questions deserving of consideration in the present symposium is whether improvements in analytical processes during the last half century justify a modification in the old Henneberg scheme of valuation.

Henneberg in the passage previously cited called the nitrogen-free extract a mixture of substances and for many years it was one of the main purposes of the school of agricultural chemistry at Göttingen, where Henneberg worked, to investigate the nature of the various substances that compose the so-called extract. A summary of the work on this subject was published in 1897 by Tollens,[6] Director of the Göttingen laboratory for agricultural chemistry. The groups of possible nitrogen-free extract substances that Tollens enumerated comprise the sugars (as sucrose, maltose, glucose, fructose, etc.), the polysaccharides (as starch and inulin), the hexosans (as dextran, mannan, galactan, etc.), the pentosans (as xylan and araban), the methyl pentosans, the sugar alcohols (as mannitol, sorbitol, etc.), the pectins, lignin, the organic acids (citric, malic, tartaric, etc.), the tannins, the vegetable colors (chlorophyl, xanthophyl, etc.), resins, and other aromatic substances. Obviously the determination of each one of these nitrogen-free extract substances would involve excessive demands on the analytical staff of the laboratory, to say nothing of the increase in labor of calculations when the results of such analyses are applied.

Henneberg realized that crude fiber and even pure cellulose, originally assumed to be indigestible, were susceptible to attack in the digestive tract of herbivorous animals. It was found on the other hand that the nitrogen-free extract, originally assumed to be completely digestible after the manner of starch and sugar, contained substances, such as dissolved lignin, of low digestibility. Comparison showed that the sum of the digestible cellulose and of the digestible nitrogen-free extract agreed approximately with the analytical results for nitrogen-free extract, a coincidence that was

[6] *J. Landw.*, 45, 295–334 (1897).

correctly characterized by the term "compensation."* This balancing of errors, however, did not always occur, there being some exceptions to the working of any such rule.

As digestion investigations were continued it was discovered that a considerable part of the loss of cellulose in the digestive tract of the animal resulted from a decomposition by intestinal microorganisms with formation of methane and carbon dioxide, as in the familiar marsh-gas fermentation, and the question arose as to whether the cellulose thus decomposed has the same nutritive value for the animal as the digestible part of the nitrogen-free extract. Obviously the escape of a part of the cellulose as methane does not represent complete digestive utilization. It has been pointed out, however, that starch and perhaps other carbohydrates of the nitrogen-free extract may also produce methane, which was another evidence of the operation of compensating influences. It was therefore concluded by Tollens that the digestive part of the crude fiber has the same nutritive value as the digestive part of the nitrogen-free extract, and since the sum of these was found to agree in general with the determination of nitrogen-free extract by the Henneberg, or Weende, method, that the practical value of this method of feeding stuff valuation was not seriously affected. The extent to which compensating influences of this kind are valid is indicated in Table 1 which is taken from the article by Tollens. It will be noted that for the 16 feeding stuffs listed the average difference between the percentage of nitrogen-free extract as determined by the Weende or Henneberg method and the sum of the percentages of digestible crude fiber and digestible nitrogen-free extract is +0.94 per cent, the range extending from +0.17 for potatoes to +11.60 for cornstalks. The agreement, while only approximate, has seemed sufficiently satisfactory to many authorities to warrant the continuance of the Weende method of analysis and valuation until some simple scheme that is more exact can be devised.

Following the work of Tollens and his pupils at Göttingen between 1880 and 1910, the determination of pentosans, as calculated from the yield of furfural, has frequently been included in the routine analysis of foodstuffs. While the pentosans, xylan and araban, are the chief furfural-yielding compounds of vegetable tissues, there are other plant constituents, such as pectin and uronic acids that also yield furfural so that the calculation of pentosans from the amount of furfural produced by distillation with hydrochloric acid, gives results that are too high. The determination is, therefore, in the same category as that of crude fat, crude protein, and crude fiber. The values are only approximations and have not the exactness that is obtainable in analyzing inorganic products, such as alloys, minerals, and salt mixtures.

One of the earliest attempts made in the United States to expand the

* This point is discussed in a review of Henneberg's work, *J. Landwirths.*, 28, 520 (1890).

TABLE 1.—*Digestibility of crude fiber and nitrogen-free extract of feeding stuffs*

1	2	3	4	5	6	7
SUBSTANCE (Dry)	RESULTS OBTAINED BY THE WEENDE METHOD PER CENT OF SUBSTANCE		DIGESTIBLE MATTER DETERMINED BY FEEDING EXPERIMENTS PER CENT OF SUBSTANCE		SUM OF DIGESTIBLE CRUDE FIBER AND DIGESTIBLE NITROGEN-FREE EXTRACT (SUM OF COLS. 4 AND 5) PER CENT OF SUBSTANCE	DIFFERENCE COL. 3—COL. 6
	CRUDE FIBER	NITROGEN-FREE EXTRACT	CRUDE FIBER	NITROGEN-FREE EXTRACT		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Orchard grass	27.02	52.75	15.93	35.34	51.27	+1.48
Wheat in shock	25.75	46.91	16.74	31.89	48.63	−1.72
Corn fodder	28.63	53.80	16.03	34.43	50.46	+3.34
Meadow grass	33.12	43.90	20.20	29.85	50.05	−6.15
Clover grass	30.27	42.00	16.65	29.40	46.05	−4.05
Beet leaves	14.35	41.86	8.32	31.40	39.72	+2.14
Medium meadow grass	29.24	49.74	17.54	31.83	49.37	+0.37
Mountain hay	27.12	53.31	16.81	35.18	51.99	+1.32
Wheat straw	43.00	45.00	23.65	17.55	41.20	+3.80
Oat straw	43.04	44.92	24.96	22.91	47.87	−2.95
Corn stalks	31.98	53.07	17.59	23.88	41.47	+11.60
Pea vines	41.03	39.04	16.01	21.48	37.49	+1.55
Potatoes	2.75	83.86	1.51	82.18	83.69	+0.17
Beets	7.68	72.15	4.22	69.26	73.48	−1.33
Wheat	2.19	79.71	1.10	75.72	76.82	+2.89
Corn	2.55	78.53	1.28	74.60	75.88	+2.65
Average	24.36	55.03	13.66	40.43	54.09	+0.94

TABLE 2.—*Analysis of wheat bran and of the feces of a steer fed thereon (Sherman)*

	WHEAT BRAN	FECES	PER CENT DIGESTED
	per cent	*per cent*	
Soluble carbohydrates as dextrin	7.2	0.7	96.9
Starch	17.7	0.0	100.0
Free pentosans	17.5	18.7	66.2
Cellulose	8.5	20.2	24.8
Lignin and allied substances	11.6	23.2	36.7
Protein	20.49	11.04	82.96
Ether extract	6.92	12.52	42.73
Ash	6.05	11.04	42.21
Undetermined	4.04	2.60	
Nitrogen-free extract	55.59	41.93	76.08
Crude fiber	10.96	23.47	32.21

Henneberg scheme of analysis so as to include other components of nitrogen-free extract was made by H. C. Sherman[7] upon wheat bran in 1896. Sherman's analyses of the wheat bran and of the feces of a steer fed upon this bran are given in Table 2.

As Sherman pointed out, a part of the lignin substance in Table 2 is included in the nitrogen-free extract and a part in the crude fiber.

The reduction of undetermined matter in these analyses to 4 per cent was a marked step in advance and even at the present day, 42 years since the publication of Sherman's results, we are unable to obtain any closer approach to the 100 per cent figure. But as Sherman clearly pointed out the factors for calculating protein from the nitrogen content and pentosans from the yield of furfural are purely conventional so that absolute accuracy in the summation of the various constituents is not to be expected.

In 1899, Browne and Beistle[8] applied a modification of Sherman's scheme to the analysis of a sample of dried distillers' grains. The summation of constituents obtained by these analysts is shown in Table 3.

TABLE 3.—*Analysis of dried distillers' grains (Browne and Beistle)*

	per cent
Moisture	3.83
Crude fat	10.25
Dextrin (?)	2.13
Starch (?)	2.66
Lignin bodies	11.95
Cellulose	15.05
Pentosans	24.86
Protein	23.44
Ash	1.84
Total	96.01

Doubts were thrown upon the determination of dextrin and starch from the fact that 3.88 per cent of pentosans was removed in the preliminary extraction with water and diastase solutions, the pentosans thus removed being afterward hydrolyzed to sugar and estimated as dextrin and starch. The amount of pentosans removed at each stage of the analytical process is shown in Table 4.

No doubt the nature of the furfural-yielding constituents varied greatly in each one of the extracts removed by the different reagents in Table 4. The results indicate the uncertainty that exists in many of our current processes of food analysis.

CONCLUSION

As a summary of the present survey it may be said that while the present methods for determining lignin, hemicelluloses, polysaccharides, pec-

[7] *J. Am. Chem. Soc.*, 19, 291–310 (1897).
[8] *J. Am. Chem. Soc.*, 23, 229–236 (1901).

TABLE 4.—*Pentosans removed by reagents at each stage of analysis of distillers' grains*

	PER CENT	PER CENT OF TOTAL PENTOSANS
Removed by alcohol and water digestion	2.70	10.86
Removed by malt extract digestion	1.18	4.75
Removed by sulfuric acid digestion	17.66	71.04
Removed by sodium hydroxide digestion	2.45	9.85
Pentosans in residual crude fiber	0.87	3.50
Total	24.86	100.00

tin, uronic acids, and other organic constituents of the nitrogen-free extract of foods and feeding stuffs enable the analyst to obtain a much more accurate idea of their composition than was possible 25 years ago, these methods, considered quantitatively, can be regarded as giving only approximate results. Moreover, because of the complicated and time-consuming character of some of these determinations, it is probably inadvisable at present to make any extensive changes in the old Henneberg scheme for the ordinary routine of feeding stuff analysis. But for the more exact purposes of research use should be made of the latest improved methods for ascertaining the composition of that complex residuum of undetermined matter that has been grouped so long and so loosely under the misleading term nitrogen-free extract.

LIGNIN AS A CONSTITUENT OF NITROGEN-FREE EXTRACT

By MAX PHILLIPS (Bureau of Agricultural Chemistry and Engineering, Industrial Farm Products Research Division, U. S. Department of Agriculture, Washington, D. C.)

The woody portions of plants such as cobs, hulls, stalks, leaves, roots, trunks of trees, and shrubs are composed principally of carbohydrates, mostly cellulose and hemicelluloses, and a complex designated as lignin. Schulze (54) is generally credited with having introduced this term into chemical literature, although it had previously been employed by the botanist and plant physiologist de Candolle (10).

Although lignin has been studied by chemists for about a century, much of the chemistry of this substance is still imperfectly understood. Much progress has been made in recent years in the studies concerning the structure of lignin, and much more remains to be done. From time to time various investigators have proposed probable structural formulas for lignin, but most of these have little definite chemical evidence for their support. This is primarily due to the fact that no method has been developed for the isolation of lignin in a pure state, and there is no method

known by which its purity may be gaged. Lignin is an amorphous substance ranging in color from light tan or brown to black, depending on the method used for its isolation, and the usual criteria of purity, such as melting point, boiling point, etc., cannot be applied.

Payen (36) first attempted to bring about a separation of lignified materials into their component parts. He showed that when treated with caustic soda, potassium hydroxide, or nitric acid, wood and other plant materials afford a relatively resistant fibrous substance, which he called cellulose. This material has the same chemical composition irrespective of its source. He pointed out also that this chemical treatment removes a product having a higher percentage of carbon than the residual cellulose. The substance or substances that could thus be separated from the cellulose by means of chemical reagents he designated "incrusting materials" (*"les matières encrustantes"*), and assumed that in lignified substances the cellulose was surrounded or impregnated with this material. Payen's "incrustation hypothesis" found support among several chemists, among them Schulze. Other investigators, notably Erdmann (12), Lange (27), Hoppe-Seyler (23), Cross and Bevan (7), Grafe (18), and others, opposed Payen's views and presented evidence to support their claim that lignin is chemically combined with the cellulose. This controversy is by no means settled, and in recent years Wislicenus (56) and Freudenberg (16) have come out in favor of Payen's views, while Phillips (37), Harris, Sherrard and Mitchell (19), and Norman (32) have presented evidence to show that lignin is found in some chemical combination with the carbohydrates of the cell-wall.

Formation. The literature contains many suggestions concerning the nature of the parent substance and the possible mechanism involved in the synthesis of lignin by the plant, but they are either purely speculative or are based on indirect or fragmentary evidence.

Klason (26) believed that lignin is formed by the plant from pentoses or methyl pentoses and that coniferyl alcohol (or coniferyl aldehyde) is an intermediate product in the process.

Rassow and Zschenderlein (48) suggested that since plant materials high in lignin are low in pentosans, and vice versa, lignin, at least in part, may be built up by the plant from pentosans. According to Odén (33) pentoses are first converted into methyl pentoses, and these are then condensed to form benzofurane derivatives from which lignin is synthesized.

Cross and Bevan (8, pp. 180–181) advanced the hypothesis that lignin is formed by the plant from cellulose. They state: "The process of lignification consists in a series of progressive and intrinsic modifications of a cellulose or oxycellulose tissue, the products of modification remaining associated with the residues of the parent substance in a state of combination or of intimate mixture, the final products of metabolism (aromatic products, pentosans, and c.) being excreted and taking no further part in

the organic processes of the tissue." Among others, they presented the following argument in favor of their theory of lignification: "Regarding lignification as a process of continuous modification of cellulose, and the woods as representing the extreme limits of such a process, these should show an increase in lignone at the expense of cellulose, which is in fact the case. Lignocelluloses in the first year of growth contain 70–80 per cent cellulose; the woods, on the other hand, 50–60 per cent."

According to an older view of Wislicenus (56), lignification is a process whereby the sum total of the dissolved hydrosols in the cambial sap is deposited upon the cellulose fiber. In a more recent publication (57), however, he suggested that while glucose is utilized by the plant in the formation of cellulose, fructose is used similarly in the synthesis of lignin.

The possibility of a biogenetic relationship between pectin and lignin has been considered by several investigators. It was observed by Candlin and Schryver (4) that pectin is found rather abundantly in young and nonlignified tissues, while plants rich in lignin are relatively free from pectin. Ehrlich (11) isolated from hydropectin of flax a fraction that resembled lignin in certain respects, and he accordingly suggested that pectin is the precursor of lignin. The evidence for this, however, is not convincing.

Buston (3), in his studies of the process of lignification of the rose plant, proves quite definitely that lignin is not produced at the expense of the pectin. While in the later stages of the development of the rose shoot, the lignin content increases considerably, the absolute quantity of the pectin does *not* decrease, but actually increases. He concludes, therefore, that "lignin does not arise from pectin, but more probably from some carbohydrate related to the glucosanxylan series with members of which series it is usually associated in the plant."

According to Zherebov (58), primary lignin contains no methoxyl groups, and lignification is a process whereby there is an accumulation of methoxyl groups. While it is true that lignin from a fully mature annual plant contains a higher percentage of methoxyl than does lignin from an immature plant, it has been repeatedly demonstrated in this laboratory that lignin from even a very young plant always contains some methoxyl.

From a study conducted by Phillips and associates (43, 47) at the Bureau of Agricultural Chemistry and Engineering on the composition of the barley plant and of the oat plant at successive stages of growth, it was concluded that lignin is not produced by the plant from cellulose, pentosans, and pectin, but directly from sucrose. Lignin was found to be present even in the very earliest stages of the development of the plants, although the percentages of lignin and methoxyl in the lignin increased regularly as the plant developed and matured.

Quantitative Estimation.—The numerous methods described in the literature for the quantitative estimation of lignin may be classified as direct

and indirect. In the indirect methods either some characteristic group of lignin, such as the methoxyl, is determined and by multiplication with a suitable factor the percentage of lignin is computed, or use is made of some color reaction or chemical property that is characteristic of lignin. The indirect methods have proved to be unsatisfactory and are hardly ever used now. In the direct methods lignin is separated from the cellulose and other carbohydrates associated with it and then weighed. The reagents most commonly used, 72 per cent sulfuric acid and fuming hydrochloric acid (42–43 per cent HCl), degrade and dissolve cellulose and hemicelluloses in the cold, leaving the lignin as an insoluble residue. The plant material is first freed of fatty, waxy, and resinous materials by extraction with an organic solvent (usually a minimum-boiling ethanol-benzene solution). Some analysts, for reasons that will be explained later, also give the plant material a preliminary extraction with hot water and with boiling dilute acids. In the determination of lignin in wood or in other plant material containing tannins of the catechol group, it is necessary to extract the sample in a Soxhlet extractor with 95 per cent alcohol for 4 hours, prior to the alcohol-benzene extraction (51). According to the modified 72 per cent sulfuric acid method proposed by Ritter, Seborg, and Mitchell (49) of the U. S. Forest Products Laboratory, the extracted and dry sample is treated with 72 per cent sulfuric acid at 20° C., and maintained at that temperature for 2 hours. The reaction mixture is diluted with a sufficient quantity of water to make a 3 per cent acid solution and boiled for 4 hours under a reflux condenser. The lignin is then filtered off, washed free of acid, dried, and weighed. If necessary to obtain the percentage of ash-free lignin, the percentage of ash in the lignin may be determined, and the proper correction applied.

In the determination of lignin by the modified fuming hydrochloric acid method developed by Goss and Phillips (17), the finely ground sample (previously extracted successivly with a minimum-boiling ethanol-benzene solution, hot water, and boiling 1 per cent hydrochloric acid solution) is treated with cold fuming hydrochloric acid, thoroughly mixed, and allowed to remain at +8 to +10° C. for 24 hours. It is then diluted with water to make a 4–5 per cent acid solution and boiled under the reflux condenser for 1 hour. The residual lignin is filtered off, the percentages of ash and nitrogen in the lignin are determined, and the percentage of lignin is calculated.

From the above summary it might be concluded that the determination of lignin is a comparatively simple matter. Unfortunately that is not the case. In fact, a quantitative method that would be applicable to all types of lignified plant materials has yet to be developed. Any method based on weighing a "residue," as is done in the case of the 72 per cent sulfuric acid and the fuming hydrochloric acid methods, can not be considered satisfactory. Among the possible sources of error may be mentioned (1)

incomplete hydrolysis of the carbohydrates, (2) formation of reversion products from the carbohydrates, (3) removal of some portion of the lignin as a result of the preliminary treatment with alcohol-benzene solution, hot water, and boiling dilute acids, and (4) contamination of the lignin residue with nitrogenous complexes.

The incomplete hydrolysis of the carbohydrates generally associated with the lignin in the plant material can result only when the sample has not been ground sufficiently fine or when the sample has not been well stirred with the strong mineral acid used in the determination. In either case there is danger of the formation of large particles having outer protective coverings of lignin or some other material equally resistant to the action of 72 per cent sulfuric acid or fuming hydrochloric acid, while the inner portion of the material may be entirely unhydrolyzed. Another error may arise when the mineral acid used is below the specified strength. This may happen particularly when the fuming hydrochloric acid method is used. Therefore, it is very important that the supply of fuming hydrochloric acid be stored in a room having a temperature of 0° C. or below. It is also desirable to determine the specific gravity of the fuming hydrochloric acid from time to time.

Difficulty may be experienced in the determination of lignin in eucalyptus woods by the usual 72 per cent sulfuric method owing to the presence of gum-like or "kino-like" substances, which are very resistant to hydrolysis. To overcome this difficulty, a rather elaborate pretreatment has been proposed involving the successive extractions with 1:2 alcohol-benzene solution, cold water, hot water, and hot 3 per cent sodium sulfite or 0.5 per cent sodium hydroxide solution, and finally hydrolysis with boiling 3 per cent sulfuric acid (5).

For many years it has been known that under certain conditions humin-like products are formed as a result of the action of strong mineral acids on sugars. Paloheimo (35) called attention to the fact that fructose and sucrose afford insoluble products when subjected to the action of strong mineral acids such as are used in the determination of lignin. Norman and Jenkins (29) show that xylose, arabinose, or substances yielding these sugars on hydrolysis, as well as sucrose and fructose, when treated with 72 per cent sulfuric acid for 16 hours at 20° C. or less and then with the usual boiling 3 per cent sulfuric acid, afford some insoluble materials as resistant as lignin. These investigators have accordingly suggested that the sample for analysis be given a preliminary extraction with boiling 5 per cent sulfuric acid in order to eliminate the interfering pentose-containing substances.

Hilpert and Littman (22) reported that they obtained relatively high yields of insoluble humin-like materials when arabinose, xylose, glucose, mannose, fructose, sucrose, starch, and inulin were treated with 72 per cent sulfuric acid or fuming hydrochloric acid. However, the experi-

mental conditions of temperature and reaction period used differed somewhat from those usually prescribed. Bamford and Campbell (1) found that xylose, fructose, and sucrose treated with 72 per cent sulfuric acid at 10° C. for 5 hours yielded only negligible quantities of insoluble residues.

Phillips and Goss (44) studied the action of cold 42–43 per cent (fuming) and 5 per cent boiling hydrochloric acid on various carbohydrates in relation to the determination of lignin by their method and found that arabinose, xylose, glucose, mannose, galactose, maltose, starch, and cellulose, either alone or in the presence of lignified cellulosic material, do *not* afford insoluble humin-like precipitates and would, therefore, not interfere with the determination of lignin in plant materials containing these carbohydrates. The only carbohydrate that might cause any interference with the determination of lignin is fructose or substances yielding this sugar on hydrolysis, such as sucrose and inulin. Accordingly, to eliminate the error likely to result in the analysis of materials that might contain fructose, these authors suggested that the sample be given a preliminary extraction with boiling water and boiling 1 per cent hydrochloric acid solution. They considered that this treatment would remove quite effectively any sucrose, fructose, fructosans, or fructosides that might be present, although they did not overlook the fact that this might also remove some fraction belonging to the lignin complex (42), but the possibility of error from this source was considered to be inconsequential. Boiling with dilute acid subsequent to the treatment with cold strong mineral acid is normally a part of the methods generally used for the determination of lignin. The recent results of Cohen and Harris (6) and Harris and Mitchell (21) indicate that preliminary extraction of wood with 3 per cent boiling sulfuric acid and even with boiling water gives low yields due to the removal of some of the lignin.

Several investigators have pointed out that whereas lignin isolated from wood by hydrolysis with either 42 to 43 per cent hydrochloric acid or 72 per cent sulfuric acid contains practically negligible quantities of nitrogen, this is not the case with lignin isolated from plants containing greater quantities of nitrogenous constituents. In determining lignin in young plants and in corn cobs, cereal straws and stalks, bran, hulls, hay and similar agricultural materials containing proteins or other nitrogenous complexes, the "lignin" residue obtained always contains a substantial amount of nitrogen. Paloheimo (34) called attention to the fact that proteins are not completely hydrolyzed by the strong mineral acids used in the determination of lignin, and accordingly he suggested that from the weight of lignin obtained should be deducted the weight of the crude protein in the lignin ($N \times 6.25$). This procedure has been followed by other investigators, although it was realized that this involves the assumption that the nitrogenous complexes associated with the lignin are of protein character (39). Norman and Jenkins (30) and Phillips (46) obtained re-

sults on the 72 per cent sulfuric acid method and on the fuming hydrochloric acid method, respectively, which show that the ratios between the increase in weight of the crude lignin due to the addition of protein and the increment of nitrogen in the crude lignin varied both with the quantity and type of protein added. In a limited number of cases, the ratios approached the figure 6.25, but in most instances this was not the case. Because of this variability, it was concluded that it is not possible to compute the ratio between the increase in the weight of the crude lignin and the increment of nitrogen in the lignin that would be applicable in all cases. No satisfactory procedure has been proposed to reduce the disturbance caused by the presence of nitrogenous complexes. Preliminary hydrolysis of the sample with boiling dilute acids has been suggested (30), but this is only partially successful in removing the nitrogenous compounds.

In the analysis of feeds and feeding stuffs by the conventional so-called Weende method, a part of the lignin remains with the crude fiber while perhaps the greatest portion is included in the "nitrogen-free extract." The percentage of lignin in the nitrogen-free extract of the more important forage plants may vary from 4 to 10 per cent. It has recently been shown by Norman (31) that the percentages of lignin and cellulose in "crude fiber" vary widely, depending upon the source from which it is derived.

Isolation.—The various methods for the isolation of lignin may be conveniently divided into two classes: (1) Those that depend on the removal, by hydrolysis, of the cellulose and other components, leaving the lignin as an insoluble residue; and (2) those that depend on the removal of lignin from the cellulose and the other substances with which it is associated. The 72 per cent sulfuric and fuming hydrochloric acid methods, which were mentioned in connection with the quantitative estimation of lignin, are in the first class. Other methods in this class are those of Urban (55) and Freudenberg (15). In place of the fuming hydrochloric and 72 per cent sulfuric acids Urban substitutes a solution of hydrochloric acid (d. 1.18) in phosphoric acid (d. 1.7). In the Freudenberg method for the isolation of lignin, the plant material is subjected to alternate treatment with boiling 1 per cent sulfuric acid and cold cuprammonium solution.

Among the methods belonging to the second class is the sodium hydroxide method, in which advantage is taken of the acidic or, more properly, phenolic character of lignin. This is the basis of the industrially important so-called soda process for the delignification of wood and its conversion into paper pulp. From the alkaline extract, upon acidulation, lignin is precipitated as "alkali lignin."

Other methods for the separation of lignin make use of various alcohols, phenols, and certain tautomeric substances. The plant material is

heated with the organic solvent in the presence of a catalyst, which is generally hydrochloric acid. In all cases a product is obtained containing the alkyl or aryl group, as the case may be, in combination with the lignin. This is in some respects a disadvantage, but in view of the fact that a rather mild treatment is employed for the isolation of the lignin as compared with some of the other more drastic methods, the lignin prepared by these methods has, in recent years, been frequently used in studies concerning its structure. Among the various compounds that have been employed for the isolation of lignin by the method referred to above are the following: methanol, ethanol, n-butyl, isobutyl, and amyl alcohols; ethylene glycol; monomethyl ether of ethylene glycol; glycerol-α-monochlorhydrin; acetic acid; and dioxane. The yields of lignin derivatives obtained by the above methods, under the conditions generally used, are relatively small, since only a portion of the lignin is thus removed.

Although never used for the isolation of lignin as such, the sulfite method should also be mentioned because it is the basis of the industrially very important sulfite process for the production of pulp from wood. The delignification reagent used is a solution of calcium bisulfite and sulfurous acid. By this method lignin is converted into water-soluble sulfonic acids and sulfonates, and the cellulose is left in a more or less pure state.

Constitution or Structure.—Lignin contains carbon, hydrogen, and oxygen in proportions that vary to some extent with the source of the lignin and the method used for its isolation. However, because lignin is amorphous, it is not at all surprising that some difference in the percentage composition is found. The figures—63 per cent carbon, 6 per cent hydrogen and 31 per cent oxygen—may be said to represent the approximate percentage composition of lignin, and it is thus seen that lignin is considerably richer in carbon than are the carbohydrates.

Constituent Groups.—That the $-OCH_3$, or methoxyl group, is present in lignin has been definitely established, and it can be quantitatively estimated by the Zeisel method. The actual percentage of methoxyl varies somewhat with the source and method employed for the isolation of the lignin. In the case of lignin from corn cobs or from cereal straws and hulls, the methoxyl content is about 15 per cent; in that from certain species of wood it is somewhat higher. In isolated lignin the presence of hydroxyl groups is clearly indicated in acylation and alkylation experiments. There is also considerable evidence to indicate that alcoholic, as well as phenolic or enolic, hydroxyl groups are present (2, 41).

The fact that various lignified plant materials, when distilled with dilute sulfuric acid or digested in the cold with sodium hydroxide, yield acetic acid or sodium acetate, respectively, while cellulose treated under similar conditions does not yield these products, has been accepted by certain investigators as evidence of the presence of the acetyl group in lignin.

However, it has recently been demonstrated that in the case of woods, at least, the acetyl group is associated with the carbohydrates and is not a part of the lignin complex (50).

Freudenberg (14) contends that lignin contains the methylene dioxide group (-O-CH_2-O-), but this has been challenged by other investigators (40, 24). From all the evidence, it would appear that the presence of the methylene dioxide group in lignin is extremely doubtful.

Besides the groups already mentioned, a number of investigators have assumed the presence in lignin of carbonyl, either in the form of an aldehyde or keto group, but none of the evidence is conclusive. Aromatic hydrazines have not given products that lend themselves readily to identification. The evidence as to the presence or absence of the ethylenic bond in lignin is inconclusive (28).

No extended discussion and criticism of the various views concerning the structure of lignin will be attempted. There continues to be considerable difference of opinion as to whether lignin belongs to the aliphatic, aromatic, hydroaromatic, or heterocyclic series.

When lignin is fused with potassium hydroxide two aromatic substances, namely, protocatechuic acid and catechol, are obtained. In 1931, the writer (38) first isolated and identified n-propyl guaiacol as one of the degradation products of lignin from corn cobs. In this compound the hydroxyl group, methoxyl group, and n-propyl side chain are in the positions 1, 2 and 4, respectively, in the benzene ring. Harris, D'Ianni and Adkins (20) and Hunter, Cramer, and Hibbert (25) recently confirmed this finding with respect to the three-carbon side chain. This does not necessarily exclude the possibility that other groups of the aromatic series may be present in lignin. There can, however, be little doubt that lignin has either an aromatic nucleus (or nuclei), which in addition to other substituents has a side chain of at least three carbon atoms, or one that may readily be converted into an aromatic substance under the prevailing experimental conditions.

Microbial Decomposition and Digestibility in the Animal Body.—Considerable work has been done in recent years on the microbial decomposition and digestibility in the animal body of lignin and lignified materials. No extended review of this work will be attempted, since these investigations form the subjects of two separate papers in this symposium. However, a few observations from one who is primarily interested in the chemistry of lignin may be given.

There is considerable difference of opinion as to whether lignin is or is not decomposed by bacteria and fungi in general. Certain investigators in the past have failed to realize that isolated lignin is not identical with the lignin that occurs naturally in the plant, at least insofar as the action of microorganisms is concerned. Lignin isolated by either the fuming hydrochloric or 72 per cent sulfuric acid method appears to be quite resistant

to the action of soil microorganisms and to certain of the wood-destroying fungi (53), but certain fungi, e.g., *Fomes pini* (also known as *Trametes pini*), have the capacity of removing and apparently of utilizing the lignin of wood and producing what is known as white-pocket rots. In a recent chemical examination of the sporophores of *F. pini*, it was found that when they were treated with fuming hydrochloric acid, under the experimental conditions usually prevailing for the isolation of lignin from higher plants, a lignin-like substance was obtained to the extent of 46 per cent of the starting material. This substance, unlike lignin, contained no methoxyl groups, although in some of its chemical reactions it resembled lignin (45).

Because there is considerable evidence that the lignin occurring in the plant is relatively resistant to microbial attack, at least under anaerobic conditions, certain investigators have considered that lignin contributes largely, though perhaps not exclusively, to the formation of humus in the soil. This is also the basis of Fischer's (13) hypothesis of the origin of peat and coal.

Although lignin is a constituent of many of the plant materials used as food by man and animals, the metabolism of this substance has not been studied extensively. Some investigators claim that lignin is in part digested by certain animals, while others deny this (52). There are data showing that when alkali lignin was fed to cows or dogs there was an increase in the benzoic acid (as hippuric acid) eliminated in the urine, the benzoic acid apparently coming from the aromatic portion of the lignin molecule. The lignin was also in part demethoxylated (9). One reason, perhaps, for the lack of agreement among the investigators may be attributed to the different analytical methods used. Moreover, much of the information available was obtained when the limitations and shortcomings of the methods were not fully realized.

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THE HEMICELLULOSE CONSTITUENTS OF THE NITROGEN-FREE EXTRACT

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The hemicelluloses are widely distributed in the vegetable kingdom and, next to cellulose, are undoubtedly the most abundant of the materials of plant origin. Hemicelluloses may be defined as carbohydrate substances that, in their natural state, are insoluble in boiling water but are soluble in dilute aqueous solutions of the alkalies and, upon heating with dilute acids at atmospheric pressure, afford simple sugars and frequently also uronic acids.

The term hemicellulose was introduced into chemical literature in 1892 by E. Schulze (24), who applied it to a product that he obtained from leguminous seeds, wheat bran, and other materials derived from cereals by extraction with alkali and precipitation of the extract with acid. This product, unlike cellulose, could be readily hydrolyzed with boiling dilute acid and could be destroyed by means of the so-called F. Schulze's reagent. Cellulose, on the other hand, was relatively resistant to this treatment.

In connection with certain types of hemicelluloses two terms have recently come into use, namely, "polyuronides" and "cellulosans." The term "polyuronides," introduced into chemical literature by Candlin and Schryver (6), refers to a group of substances, widely distributed in the plant kingdom, which are formed by the conjugation of certain sugar acids (glucuronic and galacturonic acids) with sugars. While most of the hemicelluloses are polyuronides, it must be emphasized that not all polyuron-

ides are hemicelluloses, for pectins, gums, and mucilages also contain uronic acids. The term "cellulosans" refers to those hemicelluloses that are usually free of uronic acid and are intimately associated with the cellulose. Thus the pentosan fraction of Cross and Bevan cellulose is a "cellulosan" (12). At present the term hemicellulose is used in the plural to indicate that not one substance, but rather a group of closely related substances is involved.

Origin and Role in the Plant.—There is speculation regarding the origin of the pentoses in the hemicellulose complex and the role of the hemicelluloses in the economy of the plant. Tollens (29) thought that the pentoses are formed by the atmospheric oxidation of starch or cellulose with the aid of ferments. Spoehr (25) advanced the hypothesis that pentoses are formed by the plant by decarboxylation of uronic acids, the latter being formed by the oxidation of the terminal $-CH_2OH$ group of the hexose sugar. It is interesting to note that in nature d-xylose is usually associated with d-glucose and d-glucuronic acid, while l-arabinose is generally found with d-galactose and d-galacturonic acid. From an examination of the configurational formulas of d-glucuronic and d-galacturonic acids, it can be seen that the former on decarboxylation would yield d-xylose, while the latter would give l-arabinose.

There is considerable evidence to indicate that in certain plants, at least, the hemicelluloses serve as a reserve food and are utilized by the plant during periods when other food substances are not readily available or cannot by synthesized (25). It was formerly held that the hemicelluloses are present in the plant as encrustants and are not combined with any of the plant constituents. Evidence recently presented, however, indicates that the hemicelluloses do not exist in the free state, but are combined chemically with the lignin (11).

Preparation and Fractionation.—The various hemicellulose preparations described by the earlier investigators are heterogeneous mixtures of hemicelluloses and lignin, pectins, organic acids, and other substances that might be removed from the plant material under the prevailing experimental conditions. In some cases no attempt was made actually to isolate the hemicelluloses, but the plant material, previously freed of sugars and pectins, was hydrolyzed with dilute acid and the sugars in the acid extract were determined. Thus Frear, Carter, Browne (10) and associates at the Pennsylvania Agricultural Experiment Station, in their studies on the composition of timothy hay, first removed the sugars by extraction with 85 per cent ethanol; then extracted the residual material successively with water at 50° C. and with cold 2 per cent sulfuric acid, and finally boiled for 15 hours with 6 per cent sulfuric acid.

Browne (4) isolated a crude hemicellulose preparation from sugarcane fiber (bagasse) by treating the plant material (previously freed of sugar by repeated extraction with water) with 5 per cent sodium hydroxide solution, concentrating the alkaline extract to one-third of its original volume

and precipitating the hemicelluloses by the addition of two volumes of ethanol.

Before the hemicelluloses are isolated it is desirable to extract the plant material with a 1:2 alcohol-benzene solution in order to free it from fatty, waxy, and resinous materials; organic acids, etc. Certain plant substances rich in tannin should be extracted with 95 per cent ethanol prior to the alcohol-benzene extraction. The material should then be extracted successively with hot water to remove sugars and with a hot (85° C.) 0.5 per cent ammonium oxalate solution to free it of sugars, pectin, and protopectin, respectively. Some investigators have digested the plant material in the cold with a 2 per cent solution of ammonia. In this way it is claimed that tannins, coloring substances, and resins are removed. However, it has never been definitely established that the ammonia does not remove any fraction that might be considered as properly belonging to the hemicelluloses.

A few investigators give the plant material a preliminary extraction with either cold or boiling alcoholic sodium hydroxide solution in order to reduce the quantity of lignin in the main extract and to facilitate the subsequent removal of the hemicelluloses. The use of hot alcoholic sodium hydroxide solution for this purpose has been criticized by Norman (16, a, c) on the ground that it removes also some of the hemicelluloses. This criticism has been answered, in part, by Angell and Norris (2).

A frequently used procedure for the purification of hemicelluloses is the so-called Salkowski (23) method, which is based on the fact that most hemicelluloses are precipitated from their alkaline solution by Fehling's solution. The bulky and gelatinous precipitate of the copper-hemicelluloses complex is filtered off, and the hemicelluloses are regenerated by treatment with dilute acid. According to Browne (4) the Salkowski method of purification results in considerable loss of hemicelluloses in the case of sugarcane fiber. Similar results were obtained by the writer and his associates (20) in their studies of the hemicelluloses of alfalfa hay and of wheat straw. According to Salkowski (23) the arabans from beets and cherry gum are not precipitated by his method.

Heuser, Braden and Kürschner (13) modified and improved Salkowski's method, and in order not to contaminate their product with lignin they used bleached straw pulp (method of delignification not given) as their starting material. The copper-hemicellulose complex was washed with 80 per cent alcohol and then suspended in 95 per cent ethanol; hydrogen chloride was passed into the alcoholic suspension until the precipitate was white. They obtained a product which, on the basis of the yield of furfural, was considered 96 per cent xylan. It is doubtful, however, whether this product was free of uronic acid and araban. This is evident from results recently obtained by Weihe and the writer (20) on the hemicelluloses of wheat straw.

Improved methods for the separation and fractionation of the hemicel-

luloses were introduced in recent years by a group of English investigators, among whom may be mentioned Schryver and co-workers (8), O'Dwyer (18), Norris (17), Preece (21), Norman (16), and Buston (5).

O'Dwyer (18, a) extracted the hemicelluloses of American white oak wood by treating the sawdust (previously extracted with hot water) with 4 per cent aqueous sodium hydroxide solution at room temperatures for two days, during which time it was well stirred with a mechanical stirrer. The alkaline extract was made acid with acetic acid and the hemicelluloses were precipitated by the addition of an equal volume of ethanol. These were then purified according to the Baker and Pope (3) modification of Salkowski's method. In a subsequent study on the hemicelluloses of beech wood, O'Dwyer (18, b) modified the above described method. The precipitate obtained by acidification of the alkaline extract with acetic acid was designated "hemicellulose A." When alcohol was added to the filtrate from hemicellulose A a second precipitate termed "hemicellulose B" was obtained. These two precipitates differed in appearance and composition, as well as in physical properties. This method of isolation of hemicelluloses by fractional precipitation has been followed with modifications and additions by most of the later workers.

Norris and Preece (17, a) in their studies on the hemicelluloses of wheat bran first extracted the material twice with a 0.5 per cent ammonium oxalate solution at 90° C. for 2 to 3 hours to remove pectin. The pectin-free material was partly delignified by two extractions with a 1 per cent solution of sodium hydroxide in 50 per cent ethanol and then for a further period of 1 hour with neutral 50 per cent ethanol. The hemicelluloses were extracted by several treatments with cold 4 per cent sodium hydroxide solution. The filtered and combined extracts were made acid with acetic acid and the precipitate of hemicellulose A was separated at the centrifuge. To the filtrate from hemicellulose A half its volume of acetone was added, and the precipitate designated as "hemicellulose B" was obtained. The addition of a further volume of acetone afforded another precipitate termed "hemicellulose C." Each fraction was redissolved in 4 per cent caustic soda solution and purified by the method of Salkowski. The copper complex was in each case decomposed with acid and the hemicelluloses were regenerated. To the filtrate from the copper complex half its volume of acetone was added, and the precipitate was decomposed with acid. The hemicellulose fractions thus obtained were designated A_2, B_2 and C_2, respectively.

Although this method of fractionation has been used by a number of workers, its value has yet to be proved. While a partial separation of the hemicelluloses may result in certain cases, one should never lose sight of the fact that the basis of the method is essentially a physical one and that the hemicelluloses A, B, and C are still, without doubt, rather complex mixtures and not individual substances.

The contaminant most commonly encountered in various, or even in so-called purified, hemicellulose preparations is lignin. Most investigators failed to determine the percentage of lignin in their preparations. Hemicelluloses isolated from plant materials rich in nitrogen may also be contaminated with nitrogenous complexes.

In order to isolate lignin-free hemicelluloses from jute fiber, Chowdhury and Soha (7) used the chlorine dioxide delignification procedure of Schmidt and co-workers. While this method removes the lignin, there is good evidence that it is not without effect on the hemicelluloses. In investigating the hemicelluloses in cereal straws and stalks and in forage plants, the writer used the following general procedure and obtained hemicellulose preparations that were entirely free of lignin (20). The plant material was first successively extracted with 1:2 alcohol-benzene solution, hot water, and hot 0.5 per cent ammonium oxalate solution. The material thus freed of various extractives, pectin, and protopectin was partially delignified by digestion at room temperature with 2 per cent alcoholic sodium hydroxide solution and was then exhaustively extracted with a 5 per cent aqueous sodium hydroxide solution; and the hemicelluloses were precipitated from the alkaline solution with 95 per cent ethanol and freed from sodium hydroxide by repeated washings with 95 per cent ethanol, acidified 70 per cent ethanol, and finally with neutral 70 per cent ethanol. The hemicelluloses were then delignified by alternate chlorination and extraction of the lignin chloride with a 3 per cent alcoholic ethanol amine solution, in general accord with the procedure of Ritter and co-workers on the preparation of "holocellulose" (22). In connection with the preparation of the hemicelluloses of wheat straw, it was found that one chlorination and a subsequent extraction with the alcoholic ethanol amine solution was sufficient to remove all the lignin from the hemicelluloses. However, in other cases this operation had to be repeated two or three times before a product entirely free from lignin was obtained.

Composition.—The earlier workers considered the hemicelluloses as true polysaccharides. By hydrolysis with dilute mineral acids, xylose, arabinose, or hexose sugars were obtained and the hemicelluloses were therefore considered as arabans, xylans or, in general, pentosans or hexosans; and when both hexoses and pentoses were present, they were designated as hexo-pentosans. While there are a few authentic cases known where the evidence indicates clearly that certain hemicelluloses are polysaccharides, it is nevertheless true that most hemicelluloses are polyuronides. Much of the earlier work on the hemicelluloses was done before it was known that uronic acids occur in plants. It has been known, of course, for many years that glucuronic acid is a product of animal metabolism and in fact is part of the general scheme of detoxication mechanism of the animal body. The animal organism has the power of combining toxic substances

with glucuronic acid and excreting them in the urine. On the basis of certain color reactions, Palladin (19) concluded that glucuronic acid and glucuronides were present in plants. Later Spoehr (25) succeeded in isolating a very small quantity of the crystalline lactone of glucuronic acid from cactus gum and in 1917 Ehrlich (9) isolated galacturonic acid from pectin. In 1926 O'Dwyer (18, b) identified glucuronic acid and galacturonic acid as degradation products of the hemicelluloses of beech wood and since then these acids have been found in many hemicelluloses and in other materials of vegetable origin. Glucuronic acid and galacturonic acid are the only uronic acids thus far found in seed plants, though mannuronic acid has been shown to be present in certain marine algae. While glucuronic acid usually occurs in plants in the unmethylated form, in the case of mesquite gum it is present as the methyl ether and this ether is probably also a constituent of the hemicelluloses of mesquite wood (1).

Among the sugars that have been isolated or identified as hydrolytic products of the hemicelluloses are arabinose, xylose, galactose, mannose, glucose and fructose. Just how the sugars and uronic acid components are combined to form the various hemicellulose complexes is at present not known.

Quantitative Estimation.—No satisfactory method is at present known for the quantitative estimation of the hemicelluloses. The available methods may be conveniently divided into three types. In the first type, the plant material is first freed from sugar and starch and then boiled with dilute acid, and the reducing sugars in the hydrolysate are determined by usual methods. The result is generally expressed in terms of dextrose. Dextrose is not the sugar usually present, but there is a more serious objection to this method. The hydrolysate may consist of a mixture of hexoses, pentoses, uronic acids, and possibly other substances capable of reducing Fehling's solution. The reducing value thus obtained is the resultant of several interacting and opposing effects, and it is practically impossible to translate such a value in terms of actual quantity of hemicelluloses.

The method most commonly used by agricultural chemists for the estimation of hemicelluloses, generally called pentosans, is based on the fact that these substances when boiled with mineral acids yield furfural. This method, which was developed by Tollens and his school, is so well known that no detailed description of it is necessary. The method is essentially an empirical one and strict adherence to a fixed set of conditions is essential. In the procedure usually followed, due to Kröber (14) the furfural is precipitated with phloroglucinol, although other precipitants, such as barbituric acid, thiobarbituric acid, and 2.4-dinitrophenylhydrazine, have also been recommended (27). Various volumetric methods for the determination of the furfural have also been described. It may be stated, however, that while the method is quite satisfactory for the determination of

pure pentose sugars, there are serious objections to it when used for estimating the percentage of hemicelluloses in a plant material. The hemicellulose under investigation may consist of hexosans, pentosans, glucuronic acid, and galacturonic acid. The yield of furfural obtained from arabinose is different from that afforded by xylose, and the same is true of the yields of furfural from glucuronic acid and galacturonic acid. Hexosans, on the other hand, give practically no furfural when distilled with 12 per cent hydrochloric acid. The yield of furfural from such a complex mixture is, therefore, derived from several sources, and it is impossible to correlate the percentage of furfural thus obtained with the percentage of hemicellulose in the plant material under examination. The percentage of hemicellulose can be determined from the yield of furfural only in the case where the hemicellulose is rather simple in composition and the identity of the pentose sugar and of the uronic acid is known. Thus, for example, if the hemicellulose is a xylan-glucuronide, and previous extraction of the plant material is given to remove any pectin that may be present, it is possible to compute from the total yield of furfural and from the percentage of glucuronic acid, as determined directly by the method of Lefèvre and Tollens (15), the percentage of hemicellulose in the sample. The method of Lefèvre and Tollens, it will be recalled, is based on the fact that when a uronic acid is heated with 12 per cent hydrochloric acid decarboxylation of the uronic acid takes place, and for every mole of acid one mole of carbon dioxide is produced. However, if the composition of the hemicellulose is rather complex the method suggested above cannot be used.

The third method used for the determination of hemicelluloses is that due to Preece (21, b). In this case, the hemicelluloses are actually isolated and weighed. While the method is rather tedious and not especially accurate, at least an attempt is made to determine the hemicelluloses directly and not through some degradation product or products. It is a method that deserved further study. If a satisfactory analytical method were available, many problems in connection with hemicelluloses in its various phases would find a ready solution.

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STARCH AS A CONSTITUENT OF NITROGEN-FREE EXTRACT*

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Nearly all chlorophyl-bearing plants store a large proportion of their reserve food in the form of starch until this source of energy is needed by the plant for germination or sprouting. It is one of the primary sources of food for many land animals and is therefore an important constituent of the nitrogen-free extract of feeding stuffs. Roughages used for feeding purposes are generally low in starch, but seeds such as corn, wheat, rye, bar-

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ley, and grain sorghum and the dry matter in root crops (for example, white potatoes and sweetpotatoes) average 65–70 per cent of nitrogen-free extract, nearly all of which is starch.

There are still so many controversial points concerning starch and its chemistry that it will be possible in the time allotted to this paper to review briefly only a few phases of the subject.

Relative Feeding Value.—Since starch can be obtained in a pure state and is completely digestible, it has been used as a standard for measuring the production value of other feeds. Pioneer work in this field was conducted by Kellner (7). In his system one pound of starch is used as the net energy unit (metabolizable energy less energy lost in work of digestion), and the net energy value of any feeding stuff is expressed as the number of pounds of starch that would have the same net energy value for production purposes. In the United States net energy values are calculated as Therms (units of 1,000 large calories). According to Armsby (1) one pound of starch or equivalent has an average net energy value of 1.071 Therms. Thus, values expressed in the two systems can be converted one to the other.

Physical Properties of Starch Granules.—Starch granules from different sources vary greatly. Some of the smaller granules, for example those in dasheen starch, are only 1–2 microns in diameter while some of the larger granules, such as those in *Canna Edulus*, are over 100 microns in diameter. The granules are not regular in shape, but if they are all considered to be prolate spheroids, then the average volume of single potato starch granules calculated from measured diameters would be about 28,000 cubic microns. Corn starch granules would have a volume of about 1,000 cubic microns and dasheen starch granules about 23 cubic microns. Thus, the average potato starch granule has over 1,000 times the volume of the dasheen starch granule and the volume of many of the larger granules is well over 50,000 times that of the smaller. Considering the extreme variation in size of the granules, the wide variations in the physical properties of starches is not surprising.

When heated in water the granules first swell and finally burst. During this bursting period the granule has the appearance of a ruptured sack from which the more soluble inner portion is pouring forth. When stained with iodine this outer sack is purple in color while the inner portion is blue. This outer, less soluble portion has been named by various investigators alpha-amylose, farinose, or amylopectin, and the inner more soluble portion beta-amylose, granulose, or amylose. A great deal of research has been done and many papers have been written to prove the existence or non-existence of these components of the granule.

Quantitative evidence of the existence of more than one component in starch granules appears to have been first observed in connection with enzymic reactions. Starches were found to differ greatly in the amount of

maltose produced in malting; on the average from 70 to 80 per cent maltose and from 20 to 30 per cent dextrin were obtained. That is, from 70–80 per cent of the starch was changed to maltose rapidly while the remainder changed very slowly, the process extending over several days. Many papers appeared on this subject before the year 1900.

More recently, dispersions of starch have been made by such methods as treatment of the starch with dilute alkali, with water under pressure, or with ammonium thiocyanate; or by grinding for many days in a ball mill followed by dispersion in water. The insoluble component (alpha-amylose) was then separated by sedimentation or by sedimentation and electrodialysis. It is considered that the acid radicals present in starch are combined with the alpha-amylose fraction and would be attracted to the positive pole in a cataphoresis cell. Thus, if the positive pole is placed at the bottom of the cell, advantage may be taken of both the effect of sedimentation and of migration due to the electric current. In the fractionation of potato starch by means of these methods, the quantity of the insoluble fraction (alpha-amylose) reported by different investigators varies from 2 to 84 per cent.

An explanation of the widely varying results which have been obtained was presented by R. Sutra (12) in a recent publication. Briefly, his conclusions are that "the components designated by the name alpha- and beta-amylose are not pre-existent in the grain of starch and may not be considered as constituents of starch. The envelope and interior of the grain are composed of identical substances but of different contexture, and materials that accompany starch are products of adsorption and thus no definite combination exists between phosphoric acid and amylaceous material. Alpha-amylose is formed under the same conditions as retrograded starch. It results from the aging of starch paste; aging may be brought about rapidly by the addition of various substances. Beta-amylose is a product of the degradation of starch. Theories that explain the chemical heterogeneity of starch are supported by experimental facts but the interpretation is inexact. A chemistry of starch, properly speaking, does not exist but only a chemistry of its degradation."

Evidence in favor of at least one of the contentions in Sutra's statement was given at the Baltimore meeting of the American Chemical Society by Schoch (11), who demonstrated that fatty acids, formerly considered to be combined with starch from corn to form esters, were not so combined and were merely occluded in a heterogeneous manner in the starch granule.

Quantitative Determination of Starch in Plant Materials.—Much intensive research has been devoted to this subject, chiefly because it has been difficult to find a reagent that will react with starch and at the same time not react with any of the other compounds of the plant material being analyzed. Two official methods are outlined by the Association of

Official Agricultural Chemists. The first consists in the direct acid hydrolysis of starch, pentosans, and other easily hydrolyzable carbohydrate bodies present in the sample, followed by the estimation of dextrose and the calculation, as starch, of the material hydrolyzed. In this method the percentage of non-starch material hydrolyzed often may be greater than the amount of starch in the sample, so that misleading results concerning the starch content of plant materials have sometimes been obtained and published. In the second method malt diastase is used to dissolve gelatinized starch, after which the dissolved product is hydrolyzed with acid and the resulting dextrose determined. This method has been criticized by many investigators, who have shown that malt diastase will produce reducing sugars from polysaccharides other than starch. Walton and Coe (13) proposed a modification of the malt diastase method, in which interfering polysaccharides are removed from the components solubilized by the diastase treatment by precipitation with 60 per cent alcohol, after which the acid hydrolysis is carried out and the resulting dextrose determined.

Taka diastase is also quite widely used in the determination of starch but there is evidence to show that it also hydrolyzes non-starch material (6) and thus gives correct results only in special cases.

In 1935, Hanes (5) described a method in which beta-malt-amylase (from ungerminated barley) was used as a selective hydrolyzing agent. According to the data presented, this reagent is specific for starch. Other advantages are that it yields maltose almost exclusively and possesses a definite hydrolysis limit. Should this method prove to be specific for starches from all sources, it will clear up many doubts and inconsistencies in connection with the determination of starch in plant products. Such a method is needed as a standard, even though a considerable amount of time may be involved in making a single determination.

In 1934, Denny (2, 3) reported studies on the determination of starch in a number of plant tissues, in which starch was extracted with calcium chloride solution. The starch was then precipitated as starch iodide, the iodine and calcium chloride were removed from the starch, and the starch was then estimated with taka diastase. The results indicate that the calcium chloride-iodine treatment was selective for starch and had the merit of showing low or zero values with tissues which gave no qualitative test for starch. The official methods, the Walton and Coe modification, and taka diastase estimation showed an appreciable percentage of starch with some tissues even though the qualitative tests were negative.

Rapid methods for the estimation of starch are in demand in developmental work of various kinds; for example, in plant breeding work in connection with the development of high starch content varieties of sweetpotatoes, one laboratory has estimated that it will be necessary to make starch determinations on as many as 1,000 seedlings during a single

harvesting season. Polariscopic methods (9) have been adopted for these routine analyses and have resulted in a great saving of time. Starch determinations may also be made with a fair degree of rapidity by specific gravity methods and also by isolating and weighing the starch. Specific gravity procedures have been in use for many years in Europe for estimating the starch content of potatoes. The standard procedure is based on the fact that starch has a specific gravity of approximately 1.65 and that other solids in potatoes ground for the manufacture of starch vary in relatively direct proportion to the starch content. Thus, the starch content of potatoes is approximately in direct proportion to their specific gravity. Attempts to apply this procedure to the estimation of starch in other root crops has not been successful.

In the gravimetric methods, starch is dispersed in a suitable reagent, then filtered and precipitated and weighed. Among these methods the Rask procedure (10), in which dilute hydrochloric acid is used as the dispersing agent and alcohol as the precipitation agent, is perhaps the best known. Modifications of this method have been found to be satisfactory for the determination of starch in such products as flour, but it has been pointed out by Denny (3) that "approximately correct or entirely incorrect results may be obtained with the method dependent upon the type of tissue which is used." For example, only a small amount of the starch present in black walnut leaves was dissolved by the acid used and low results were obtained; while orange fruit rind, which gives no qualitative test for starch, showed on analysis 15 per cent of material that was reported as starch, but which was presumably pectin rather than starch.

Studies on the direct polarimetric estimation of starch were reported by Ewers (4) and by Lintner and Belschner (8) in 1906 and 1907. They used dilute hydrochloric acid as the dispersing agent. Since that time studies on the use of calcium chloride, magnesium chloride, hydrogen peroxide, and other oxidizing agents and of various enzymes have been reported in the literature. These methods are rapid, and with some of the reagents determinations may be completed in less than an hour's time. None of the reagents proposed is entirely specific for starch, that is, all may dissolve optically active substances other than starch. But, as may be seen from the brief review which has been given, the same criticism applies to all of the other methods that are in common use. By proper selection of the dispersing agent, and occasionally comparing the results obtained by a routine polariscopic method with those obtained with a reagent that is probably specific for starch, such as beta-malt amylase or the calcium chloride iodine treatment, results fully as accurate as those obtained with commonly used methods might be obtained by polariscopic methods with less expenditure of time. Dispersing agents that do not dissolve many of the plant tissue components that are often calculated as starch should give reproducible results in the determination of starch in commonly occurring plant products.

If sufficiently rapid methods for the estimation of starch were available, it would be feasible to subdivide the nitrogen-free extract content of feeds and include a starch value in the analysis reported.

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INULIN AND HEMICELLULOSE IN NITROGEN-FREE EXTRACT AND POSSIBLE IMPORTANCE OF HEMICELLULOSES IN ANIMAL NUTRITION*

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In the analysis of plant material the nitrogen-free extract is reported as such or is partially analyzed and reported as sugars (reducing and non-reducing) and starch. The undetermined remainder of the nitrogen-free extract is variously termed pentosans or hemicellulose. The quantitative methods for analyzing the latter fraction are still not wholly satisfactory and the hemicellulose of a plant is usually a subject of special investigation. The various methods for determining sugars and starch are quite satisfactory for materials whose ingredients are known and when only the variations in percentage of the different constituents are studied. When, however, we deal with plants of unknown composition, greater care should be exercised. For example in determining reducing sugar as glucose, we might, in reality, be dealing with a mixture of various monosaccharides and disaccharides, and the non-reducing sugars determined as invert sugar or sucrose may represent a mixture of various oligosaccharides and even polysaccharides. Important constituents of the plant material may,

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therefore, be entirely overlooked, which will not only leave a gap in our chemical information, but will also tend to introduce an error in the evaluation of food or feed value of the material in question. The food value of glucose, or fructose, or sucrose is well known but little is known about the food values of xylose, or of arabinose, or of inulin. The following discussion is confined to the effect of inulin, often found in plant material, and of hemicellulose, always present in plants, on the routine analysis of the nitrogen-free extract.

Inulin.—This polysaccharide is widely distributed in the vegetable kingdom, but is found particularly among the plants of the Compositae and the Lily families. As carbohydrate reserve material, it occurs in large quantities in tubers and roots of various plants (dahlia, chicory, Jerusalem artichoke, dandelion, and others). Recently published analyses of over 100 Indian food plants (17) showed that 9 per cent of the species examined contained considerable amounts of inulin, up to 60 per cent of the plant on dry basis. However, the physical properties of inulin from various sources do not seem to be identical (16). In a general way, inulin from the plants of the Lily family is more soluble than that from the family Compositae. But even preparations from different species of the same family are not identical. The less soluble inulin can be detected by placing a thin section of the plant in strong alcohol and observing the characteristic sphero-crystals under the microscope (3). Dilute acids hydrolyze inulin almost entirely to fructose (6), although some glucose is formed during the hydrolysis. It is also hydrolyzed to fructose by the enzyme inulase.

The following discussion of the determination of the reducing sugars, non-reducing sugars, and starch of the nitrogen-free extract relates to the A.O.A.C. methods recommended for grain and stock feed (9). In the method for sugars the material is extracted with 50 per cent alcohol, brought to a definite volume with 95 per cent alcohol, and filtered. The alcohol is evaporated and the sugars brought into water solution, clarified with lead acetate, and the excess of lead is removed. If inulin is present in the material analyzed, it will not affect the determination of reducing sugar unless it has been hydrolyzed to some extent, in which case the reducing sugars will contain a great deal of fructose. The entire amount of the unhydrolyzed inulin will be determined in this scheme of analysis as non-reducing sugar.

A simple procedure to give some idea of the nature of non-reducing saccharine material present consists in taking a polariscopic reading before and after inversion (17). Thus, if sucrose is the principal sugar present, its positive rotation will change on hydrolysis to the negative rotation of the mixture of glucose and fructose. This well-known fact is the basis of Clerget's double polarization method for determination of sucrose, and what follows is a qualitative extension of the same method.

Occasionally glucosides are present in the plant material. The natural beta glucosides have a negative rotation. On hydrolysis glucose, with positive rotation, results, provided that a glucone part of the molecule is optically inactive. In the case of inulin (sp. rot. about −35°), which yields fructose (sp. rot. about −92°) on hydrolysis, the negative rotation before hydrolysis will change to a greater negative rotation after hydrolysis. However, it is not often that one sugar only is found in the plant material. In the case of inulin, the presence of sucrose or glucose in moderate amounts will enhance the difference in rotation before and after inversion, and the presence of fructose will reduce the difference. The following examples illustrate the actual workings of the method. Dahlia roots are known to contain inulin. Actual analysis of one sample gave (on the dry basis) 7.3 per cent reducing sugar, calculated as glucose, and 29.1 per cent non-reducing sugar, calculated as sucrose. The rotation before inversion was −1.7° V., after inversion, −4.4° V., which shows that most of the non-reducing saccharine material is in the form of inulin.

In the plants cited below, inulin was discovered by the same method. Camas, a plant of the Lily family (*Quamasia quamash* (Pursh) Coville), is rich in inulin. Camas bulbs were widely used for food by the Indians of the Northwestern States (14). The analysis of the bulbs (from Oregon) gave 8.8 per cent reducing sugar, and 34.1 per cent non-reducing sugar. The rotation was −2.0° V. before inversion, and −5.6° V. after inversion (16), thus indicating that most of the non-reducing saccharine material was in the form of inulin, which subsequently was isolated from this plant. As another example, balsam root from Utah (*Balsamorhiza sagittata* (Pursh) Nutt. of the family Compositae) gave on analysis 2.3 per cent reducing sugar and 6.8 per cent non-reducing sugar. The polariscopic reading before and after inversion was −1.0° V. and −3.3° V., thus proving the presence of inulin, which was subsequently separated from the plant.

While inulin is determined as non-reducing sugar, its presence introduces an error during the starch determination. When the diastase method is used in presence of much inulin a high value for starch will be obtained; in fact, some value for starch might be obtained even with its complete absence in the material analyzed. Thus, 12.1 per cent (on dry basis) of starch was found in the dahlia, and 4–27 per cent in camas roots, although neither of these materials contains any starch. Such apparent values for starch are due to the difficulty in washing out the inulin from the plant material in the course of preparing it for starch analysis, due, perhaps, to the slight solubility of inulin, as in dahlia roots, or to an occluding gummy material in the plant, as in camas roots. It will also depend upon whether fresh or dried plant material is used for analysis. Fortunately, most of the inulin-bearing plants do not contain starch, but coexistence of inulin and starch in the same plant is known (4), (bulbs of snowdrop, *Galanthus sp.*, and of snowflake, *Leucojum sp.*).

Influence of Hemicelluloses on Nitrogen-Free Extract.—Hemicelluloses are complex compounds of sugar groups (xylose, arabinose, galactose, and others) with or without uronic acids. Other chemical groups can be, and are, present in the hemicellulose molecule, like methyl or acetyl (10). For the purpose of this discussion, the hemicelluloses can be divided into two groups: (1) those consisting of sugar groups only (true pentosans or hexosans) or complexes where sugar groups predominate in the molecule, and (2) complexes where the uronic acids predominate in the molecule (pectic substances). As both groups of compounds have been dealt with separately and in detail on this program, the following remarks will be confined to a few observations leading to the main topic of this paper, the effect of the presence of these substances on nitrogen-free extract.

Hemicelluloses can be extracted from the plant material either with alkalis, presumably unchanged, or with acids with accompanying hydrolysis. The presence of pectin may interfere somewhat with the extraction of the other hemicelluloses due, apparently, to partial jellying of the hydrolysis products. Thus, with beet pulp, known to contain pectin, the extraction curve (representing the extraction with increasing concentrations of acid) will rapidly reach a maximum, fall off to a minimum, and subsequently slowly rise again (15). This break in the curve is not observed when there is very little or no pectin present. Thus, for rice hulls or peanut shells a smooth extraction curve is obtained. Further proof that this break is due to pectin is furnished by the fact that beet pulp previously extracted with ammonium oxalate to remove the pectin gave a smooth curve on extraction with increasing amounts of acid.

In the extraction experiments cited 0.08 *N* hydrochloric acid at 80° C. extracted 78.1 per cent of the total furfural-yielding substances (calculated as pentosans) present in the sugar beet pulp. The same acid under the same conditions of extraction removed only 10 per cent of the total pentosans from rice hulls, and only 4.5 per cent from peanut shells, showing that the more soluble pectin goes into solution much faster than the other hemicelluloses. Even in water extraction, which is more pertinent to the subject of this discussion, this difference between the pectin-containing and pectin-free material is very considerable. At 80° C., 100 cc. water extracted from 2 g. sample 4.9 per cent of rice hulls and 6.0 per cent of peanut shells but in both cases no furfural-yielding material (i.e., no pentosanic or pectic substance) had been extracted. In the case of pectin-containing beet pulp not only is more material extracted, but much furfural-yielding substance as well. Thus, at 50° C. 12.2 per cent of the pulp was extracted; at 80°, 14.6 per cent; and at 100°, 23.7 per cent. Determination of pentosans showed that 16.5 per cent of the total pentosans were extracted at 80° C. and 24.9 per cent at 100°. If the extracted furfural-yielding material is considered primarily as pectin, the actual percentage of hemicellulosic material extracted is greater than would appear

from the figures given above, since uronic acids give a much lower yield of furfural than do pentoses. After the sugar beet pulp had been extracted with ammonium oxalate to remove the pectins and then again extracted with water at 80° C., only 3.3 per cent of pulp was extracted and no furfural yielding material at all. Another pectin-rich material is sweetpotato pulp, the by-product of sweetpotato starch manufacture. As obtained from the factory, the pulp still contains much starch (about 30–40 per cent on dry basis). The starch was removed by treatment with diastase, and the pulp thus treated lost 18.1 per cent of the material, or 16.0 per cent of the total pentosans present, on extraction with water at 80°.

In the light of these solubility relationships, the influence of the presence of pectic substances on the various determinations in the analysis of nitrogen-free extract can be traced. There is no evidence that the presence of pectic substances will affect the determination of reducing and non-reducing sugars. Some pectin might go into solution in 50 per cent alcohol, but it would probably be precipitated on further addition of 95 per cent alcohol as the method requires. There is, however, distinct evidence—and it has been recognized (12) for some time—that the presence of pectin affects the determination of starch, and that higher results are obtained in the presence of pectic substances. The pectin is not washed out during the preparation of the sample for starch analysis in the usual manner, i.e., with dilute alcohol or water at room temperature. Part of it is dissolved when the starch-containing material is heated for the purpose of gelatinizing the starch. On subsequent acid hydrolysis the pectic material will be transformed into reducing substances, which will add to the value for starch. More pectin might enter the solution during the digestion with diastase. Some diastase preparations are known to contain enzymes affecting pectin (2, 5).

Sweetpotato is a typical product containing both starch and pectin, and some work has been done to correct the higher figures for starch obtained by the usual methods of analysis. Promising methods seem to be (1) the preliminary digestion with dilute alkalis (1) and (2) calcium precipitation of the pectic substance after digestion with diastase (7). Thus, by the former method sweetpotato pretreated with 0.04 *N* calcium hydroxide gave 21.16 per cent starch, as compared with 22.32 per cent by the usual method, or a difference of 5.2 per cent. Sweetpotato pulp, the by-product of starch manufacture, still contains much starch and proportionately more pectin. The analysis of this pulp (wet) showed 5.71 per cent starch (when pretreated with calcium hydroxide) as compared with 8.35 per cent by the usual method, a difference of 31.6 per cent, and finally, sugar beet pulp, which, for all practical purposes contains no starch, gave with alkaline pretreatment 0.64 per cent starch as compared with 8.74 per cent by the usual method, or a difference of 92.7 per cent.

Hemicelluloses in Animal Nutrition.—It is well known that when some

toxic compounds are ingested, or produced as a result of faulty metabolism in an animal, they are excreted in combination with various acids. Glucuronic acid is one of these detoxifying agents. Thus, on ingestion of such substances as borneol, phenol, camphor, menthol, naphthol, and others, the amount of glucuronic acid eliminated in the urine increases considerably. Many other toxic substances behave in similar manner, e.g., administration of sulfapyridine, recently introduced into the therapy of pneumonia, causes an increased excretion of glucuronic acid (12). Assuming that the animal organism can produce only a limited amount of glucuronic acid, would it not be possible to eliminate larger amounts of toxic substances by introducing into the system glucuronic acid-containing material? Glucuronic acid is found in a number of plant materials (11). The hemicelluloses of the "roughage" quite often contain glucuronic acid in their molecules. Would it not be further possible that galacturonic acid might perform similar functions with regard to elimination of toxic substances? Galacturonic acid is the main constitutent of pectins, so widely distributed in the vegetable kingdom, and perhaps the physiological function of the hemicelluloses in general and of pectins in particular is to protect the animal organism from various toxic substances. Among the feeds of high pectin content are apple pomace, beet pulp, and sweetpotato pulp.

Manville and his collaborators (8), on the basis of experimental work with rabbits, came to the following conclusions:

1. There are apparently two sources of glycuronic acid available to the body, namely, endogenous sources synthesized from glycogenic amino-acids and exogenous sources present in food material. Very great importance must be attached to the exogenous sources under those conditions where the body demands glycuronic acid in larger quantities or at a faster rate than can be produced by endogenous metabolism.

2. Galacturonic acid is capable of forming conjugation products with toxic materials in the same manner as glucuronic acid.

3. When menthol and pectin were fed, it was noticed that as soon as the dosage of menthol increased to the point where it demanded a larger amount of glycuronic acid than could be supplied by either exogenous or endogenous sources, signs of intoxication occurred. . . . Animals show upon autopsy ulcerations in the stomach, pylorus, gall bladder, and small and large intestine. These ulcers and erosions bear a marked resemblance to those occurring in vitamin A deficiency.

This paper may be conveniently terminated by agreeing with Manville that "Foods containing hemicellulose and pectin have a value separate and distinct from caloric considerations."

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PECTIC MATERIAL AS A CONSTITUENT OF NITROGEN-FREE EXTRACT*

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Pectin material is one of the lesser constituents that make up the nitrogen-free extract of foods and feeding stuffs, although its chemical and physical properties are such as to lend considerable importance to its quality. Therefore, this discussion may well place more emphasis upon the chemical constitution of pectin, the factors affecting its quality, and the role of pectin in plant and animal nutrition, than upon mere quantity.

Definition.—The pectic substances are commonly grouped into three classes, protopectin, pectin, and pectic acid. Protopectin, which is found in growing tissue, is the water-insoluble, unhydrolyzed pectic substance that is changed to soluble pectins during growth or ripening of plant tissues, or by treatment with enzymes, acids, or other reagents in a variety of ways. The water-soluble pectins consist of a mixture of pectin units varying in size and in degree of methoxylation, termed pectinic acids when free carboxyl groups are present, and pectinates when these carboxyl groups have partially or entirely reacted with metallic salts. The water-soluble intermediate substances are converted to insoluble pectic acid through enzymic or chemical hydrolysis with a complete elimination of methyl ester groups. Pectic acid in feed stuffs, especially fruit and vegetables, is associated with over-ripe or decomposed tissues.

Constitution of Pectin.—Pectins prepared by acid extraction and alcoholic precipitation will be found to contain arabinose, galactose, galacturonic acid and acetyl and methoxyl groups. That much of this matter is extraneous and occluded by the methods of precipitation has been shown

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recently by Schneider and his associates (5), (15), (16), (17), (18). After nitrating and acetylating various pectins, they conclude that true pectin is polymerized galacturonic acid, a chain compound, free of arabinose, galactose, and acetyl groups, whose carboxyl groups are 75 per cent methylated (11.92 per cent CH_3O) and the position of the free carboxyl is arbitrary. The recent patent to Myers (9) in this country on the synthesis of pectin from uronic acids by polymerization substantiates the work of Schneider. The extent of polymerization or size of molecule varies as with cellulose, and the degree of methoxylation is not constant throughout a pectin preparation. These factors affect pectic character in various food materials.

Pectin Characteristics.—Where the average lengths of the polygalacturonic chains making up a pectin preparation are high, greater viscosities in water solution are found, and, in turn, when used in foods such as jellies, greater jellying capacities are present. Jellying values, or grades, are dependent upon quality rather than quantity.

While the extent of polymerization of the polygalacturonic acid determines quality attained by proper technic, the degree of methoxylation governs the chemical combining capacity of the pectins, which, again in turn, limits precipitation and certain jellying characteristics such as the speed of setting. However, precipitation and setting, while limited by the chemical combining capacity, are directly dependent upon the extent of the replacement of the hydrogen of the free carboxyl groups by metallic ions and by the hydrogen ion concentration of the medium.

The physical action of pectins, then, is not entirely governed by the simple change in protopectin to soluble pectins or pectinates with different degrees of demethoxylation. The differing degrees of methoxylation mean a variation in quantity of free carboxyl groups, which in turn affect the chemical combining weights of the pectin. It is the amount and kind of *available* metallic ions that determine firmness of food and vegetative tissue. If sufficient cations are available to take the place of methoxyl groups as they are split off from the pectins by chemical or enzymic action during aging, no appreciable softness of tissue develops. The most familiar cation activity is shown by calcium. The presence of monovalent cations tends to lower firmness. However, when demethoxylation takes place and there is no available favorable cation, softness develops owing to increase in solubility of the pectins. Solubility and excess available mineral constituents apparently do not exist simultaneously, although the hydrogen ion concentration appears to be a limiting factor.

Decomposition of Pectin.—Pectin is decomposed by chemical or enzymic action. When pectin solutions are heated the colloidal properties are easily destroyed. Heating causes cleavage of the long, thread-like, pectic chain into shorter chains with a consequent rapid decrease in viscosity according

to a logarithmic relationship. Reducing properties on the other hand increase in an arithmetic progression upon hydrolysis. The rate of chemical change from protopectin through pectin to pectic acid is more rapid at the higher temperatures and with an excess of acid or alkali. At low temperatures the rate of demethoxylation is more rapid than the rate of depolymerization and increases with increase in acid or alkali.

Enzymic decomposition of pectins is general throughout nature, the responsible enzymes being found in the vegetative tissues naturally or secreted by molds and fungi. Protopectin is acted upon by protopectinase. This action is associated with the ripening and softening of food tissue. Pectin is acted upon by pectase (pectin-methoxylase), which splits off methoxyl groups from pectin, forming pectinic and pectic acids. Kertesz (7) reports that pectin is also acted upon by an enzyme, called pectin-polygalacturonase, which is responsible for the decomposition and depolymerization of the polygalacturonic acid nucleus to galacturonic acid. Both of these latter enzymes, when allowed to act to completion, destroy normal jellying properties of pectins and are closely associated with spoilage of feed stuffs. A quantitative estimation of pectic material will not give any indication of the extent of decomposition.

Extraction of Pectins.—Pectins are usually extracted from fruit or vegetative tissues by mild acid or alkali hydrolysis in water solution. Time, temperature, and the hydrogen ion concentration of the extraction medium are factors in determining yields and must be considered in the development of analytical procedure for this component of the nitrogen-free extract.

As noted previously the colloidal properties of pectin are easily lost by chemical action. To illustrate the effect of a 30-minute boiling extraction of a specially prepared lemon albedo in water solutions acidified with hydrochloric acid, reference is made to the following table (10):

*p*H of Extraction	Yield	Grade (69.4% sugar)
3.0	25.2	324
2.6	32.2	350
2.4	34.4	348
2.0	37.4	260
1.5	37.5	181
0.8	28.1	48

Maximum extraction of pectin precipitable in ethyl alcohol took place at approximately *p*H 1.5, or at a concentration of less than 0.2 per cent hydrochloric acid. With an increase in acidity the yield dropped because pectin was decomposed to substances soluble in alcohol. Loss of quality takes place below *p*H 2.4. Quality, or grade, is denoted by the number of units of sugar one unit of pectin will support as a sugar jelly of a certain

soluble solids content. At *p*H 1.5, the quality of the pectin material has dropped to 50 per cent of its maximum value, but a quantitative estimation shows the greatest yield at this point.

The 1.25 per cent sulfuric acid extraction of feed stuffs used in the determination of crude fiber is a drastic one as far as pectin material is concerned. It is comparable to an extraction at *p*H 0.8, as shown above, but it does not necessarily remove all the pectic matter present. In tests upon an apple pomace containing at least 14 per cent extractable pectic material, the boiling 1.25 per cent sulfuric acid removed all but 2.77 per cent pectic acid in 5 minutes, all but 1.17 per cent in 15 minutes, and all but 0.52 per cent in 30 minutes as found by additional 30-minute boiling extractions with hydrochloric acid at *p*H 1.75. No pectic matter remained after 30 minutes of boiling with 1.25 per cent sodium hydroxide solution. The total pectin content present in a food stuff, therefore, will appear in the group of substances classified as nitrogen-free extract.

Analytical Methods.—While it should be emphasized that all pectic material now appears in analytical procedure as a constituent of the nitrogen-free extract, to evaluate quality, knowledge is required as to the quantity present. While physical measurements may be used to evaluate solution quality, quantitative chemical analysis must also be considered.

A combination of hot acid extraction of foods and feeding stuffs and precipitation of the pectins with ethyl alcohol is the simplest and commonest of the methods used for quantitative analysis, but it is of value only in comparative work where extractions and materials are similar, because starches, gums, etc., are precipitated by the alcohol and considerable amounts of other substances may be occluded. Other precipitants, such as acetone and inorganic salt solutions, are used.

The pectic acid (di-galacturonic acid) method of Wichmann (19) has been tentatively accepted by the Association of Official Agricultural Chemists. This method is based on the saponification of extracted pectin with sodium hydroxide and the conversion to pectic acid by boiling with hydrochloric acid. The somewhat similar method of Carré and coworkers (2), (3) depends upon the conversion of the saponified pectin to calcium pectate by the addition of acetic acid and calcium chloride. Estimation of pectin by titration of the acidity developed upon saponification has been suggested but cannot be termed satisfactory.

Galacturonic acid present may serve as an index of pectin content. This is determined by a modified Lefèvre and Tollens method (11) for uronic acids, which consists in measuring the carbon dioxide produced upon decarboxylation of galacturonic acid by boiling with 12 per cent hydrochloric acid.

For a true analytical picture of pectic value in addition to a knowledge of quantity and quality of the pectic matter present in food or vegetative tissue, the ash, the *p*H, and the methoxyl content should be known. The

methoxyl content may be determined by the Zeisel method (11), (12), or a modification of same, or by the saponification method of von Fellenberg (4). The Zeisel method consists of splitting off the methoxyl groups with hydriodic acid, volatilizing the methyl iodide formed, absorbing it in silver nitrate, and weighing the silver iodide precipitate. The saponification method is simpler; an excess of alkali added to the pectin solution splits off the methoxyl groups, the alkali required for saponification is determined by titrating the excess, and the methoxyl calculated.

Plant and Animal Nutrition.—The important physiological role played by pectin in plant nutrition cannot be correlated with any quantitative value. Concentration is highest in growing surfaces, but pectin has been extracted from all parts of the living plant. Its highest concentrations occur in beet pulp, apple or pear pomaces, and in the albedo of citrus fruits, where there is present a sufficient amount to warrant its commercial extraction.

Examples of quantitative yields as calcium pectate (which vary with season and age) are:

	Approx. per cent
Dry woods	8
Dry grasses	2
Dry beet pulp	3
Soft fruits, fresh berries	1
Apples, fresh	1.5
Dried pomaces	10
Dried orange albedo	20
Dried lemon albedo	30

Small root hairs of seedlings have an outer layer of pectic material which, as suggested by Howe (6), may be the medium for exchange of plant food, because pectin exhibits marked adsorption capacities in the cell wall. A change in pectic content may be associated with some disease or injury. A bruise or insect attack is followed by the growth of wound tissue that is higher in pectic substance, while a decrease in pectin content is found in apple leaves suffering from disease known as "silver leaf."

It was generally considered that as plant tissues age and become lignified the pectins are converted into lignin and hemicellulose but recent work by Anderson et al. (1) indicates that pectins are apparently present in mature wood as insoluble salts or combined with other materials just as they were laid down in the earlier stages of growth. Ruge (14) finds protopectinase will convert the water-insoluble pectin back into the active form, which will allow response of the cell to growth substance.

Pectins share with other hydrophilic colloids present in plant cells the credit for control of the ability of plants to resist cold and their suscepti-

bility to hardening. Water held by colloidal adsorption varies with an increase in colloid content and with changes in the acidity of plant tissue.

In fruits such as the apple, pectic changes take place with ripening. Protopectins change to soluble pectins, which reach a maximum concentration at the hard-ripe stage. Coincident with the formation of soluble pectin, pectic acid is forming, and as the fruit softens and passes to the over-ripe condition the decomposition products become preponderant.

The value of available favorable cations, when pectin is present, is evident in firmness of fruit and vegetative tissue. In addition to the firming of tomatoes by calcium chloride reported by Kertesz (8), the work of Personius and Sharp (13) upon cell adhesion in potato tissue is most interesting. Cell adhesion was found to be decreased by heat alone or by acids and salts, especially those chemicals capable of extracting calcium.

In animal nutrition, pectin is generally considered as beneficial to the digestive system because it is a protective colloid. Tests upon dogs show there is no increase in blood sugar after they have eaten this substance. Mixtures of intestinal bacteria decompose most pectins to galacturonic acid, but no utilizable carbohydrates have been found. Heavy metal pectinates resist intestinal decomposition.

There is a definitely favorable action of pectin preparations in the treatment of infant diarrhea, an action supposedly due to the removal of toxic substances. Pectin therapy has also been practised in the treatment of infected sores and wounds. Initial results seemed to indicate a direct effect of pectin on the bacteria present, but recent evidence points to the bactericidal action as due to the specific pectin used—a heavy metal pectinate. As in plant tissue, the availability or presence of a metal seems to be the determining factor in pectin value, and the type of metal determines activity.

Crop Residues.—Crop residues that remain in or are applied to soils determine soil organic matter. The rate of decomposition or fermentation of soil organic matter depends upon conditions that favor activity of the microorganisms, bacteria, and fungi. Although it is generally considered that pectin is readily decomposed by soil microorganisms, the latest information points to dependency upon mineral types present. Since certain of the metal pectinates have been found to exhibit a decided bactericidal action, it is possible that pectic material introduced in the soil as a component of the nitrogen-free extract of crop residues will retain its colloidal properties by resisting the action of the soil microorganisms and be the major component of the organic colloid soil fraction. Even this is subject to a gradual mineralization. The relatively low concentration of pectin matter in crop residues would indicate little value in supplying humus to the soil quantitatively.

Conclusion.—Where information on pectin is required, in addition to quantity in terms of pectic acid or pectate, quality in terms of grade,

viscosity, methoxyl content, and available ash should be given and the initial *p*H of solution indicated.

Since physical properties are most important in determining the role of pectins in feeding stuffs it would seem that any analysis showing only the amount of pectic material in the nitrogen-free extract would be of little value.

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SIGNIFICANCE OF THE CONSTITUENTS OF THE SO-CALLED NITROGEN-FREE EXTRACT OF PLANT MATERIALS AS A SOURCE OF ORGANIC MATTER IN SOIL[1]

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Any attempt to interpret the microbiological decomposition of plant material as a whole or of only one group of plant constituents, and the liberation of nutrient elements in available forms, as well as the formation of humus, must take into consideration the chemical nature of the materials undergoing decomposition. This is particularly true in dealing with such heterogeneous groups of chemical compounds as are found in the nitrogen-free extract of plant materials. It has been repeatedly established that microorganisms, even when one considers as complex a population as is commonly found in composts and in soil, do not attack the different organic compounds found in common plant materials in a similar

[1] Journal Series Paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Microbiology.

manner and with the same rate of speed. Some of these compounds are readily decomposed, others less rapidly, and still others are more or less resistant. Some are attacked under a variety of conditions and others only under certain specific environmental conditions. The decomposition of some compounds is greatly influenced by the presence of specific elements essential for the nutrition of microorganisms, especially the available nitrogen and phosphorus, whereas others are less dependent upon these elements for their decomposition.

With the advance of knowledge of the chemistry of plant materials, it became recognized that the ordinary partition of the major groups of constituents into the "crude fiber" and "nitrogen-free extract" fractions is not a sufficient measure either of their digestibility by higher animals or of their decomposition by microorganisms. It has recently been pointed out (1) that the first fraction that is supposed to be less digestible than the second may actually be as much if not more digestible. The crude-fiber determinations were found (4) to be misleading, since this fraction does not bear any definite relationship to its chemical constituents. The same may be said with even more justification of the "nitrogen-free extract" fraction.

After a number of attempts to apply the method of foodstuff analysis to peat (2), it was concluded that "the method does not give a sufficiently forceful illustration of the available organic compounds in plant remains now stored as layers of peat; it would, therefore, be preferable to differentiate between the several nitrogenous substances, carbohydrates and fats, and substitute a more accurate, though necessarily more complicated classification." The abundance of the nitrogen-free extract fraction in peat was found to vary not with the depth of peat, but with the composition of the plant materials and their stage of decomposition.

In assuming that the nitrogen-free extract fraction comprises the more readily digestible group of plant constituents, it was commonly believed that, during the treatment of the material with hot dilute acids and alkalies, only sugars, starches, and hemicelluloses are brought into solution. However, the last group comprises highly heterogeneous compounds, including various polysaccharides and polyuronides. The fact that varying amounts of lignin are also extracted by the alkali treatment tends to confuse further the significance of the fraction thus obtained.

The major criticisms recently directed against the method of foodstuff analysis has been that no consideration has been given at all to the lignin fraction. These two important constituent groups of the "nitrogen-free extract" fraction, namely the hemicelluloses and other carbohydrates and the lignins, must be taken into consideration in any attempt to study the decomposition of the fraction by microorganisms. No definite relationship between the two complexes in the "nitrogen-free extract" can be established, since their concentration varies in different plant materials and

TABLE 1.—*Chemical composition of a series of plant materials*
(On per cent of air dry basis.)

	YOUNG RYE PLANTS	MATURE WHEAT STRAW	SOYBEAN TOPS	ALFALFA TOPS	YOUNG CORN STALKS	MORE MATURE CORN STALKS	YOUNG PINE NEEDLES	OLD PINE NEEDLES	OAK LEAVES GREEN	
soluble portion	2.35	1.10	3.80	10.41‡	3.42‡	5.94‡	7.65	23.92‡	7.75	
soluble portion	29.54	5.57	22.09	17.24	28.27	14.14	13.02	7.29	22.02	1
lluloses	12.67†	26.35†	11.08†	13.14	20.38	21.91	14.68	18.98	12.50	1
ses	17.84	39.10	28.53	23.65	23.05	28.67	18.26	16.43	15.92	1
	10.61	21.60	13.84	8.95	9.68	9.46	27.63§	22.68	20.67	2
*	12.26	2.10	11.04	12.81	2.61	2.44	8.53	2.19	9.18	
	12.55	3.53	9.14	10.30	7.40	7.54	3.08	2.51	6.40	
ccounted for	97.82	99.35	99.52	96.50	94.81	90.10	92.85	94.00	91.44	8

ntosans only
her and alcohol-soluble
otein figure is obtained by subtracting water-soluble nitrogen from total nitrogen, then multiplying difference by 6.25.
e higher lignin content in the younger needles is due largely to the fact that this preparation has not been extracted with alcohol.

with the degree of maturity of the same plant. The chemical nature of these two groups of compounds will also vary, as well as the rate of their decomposition by microorganisms.

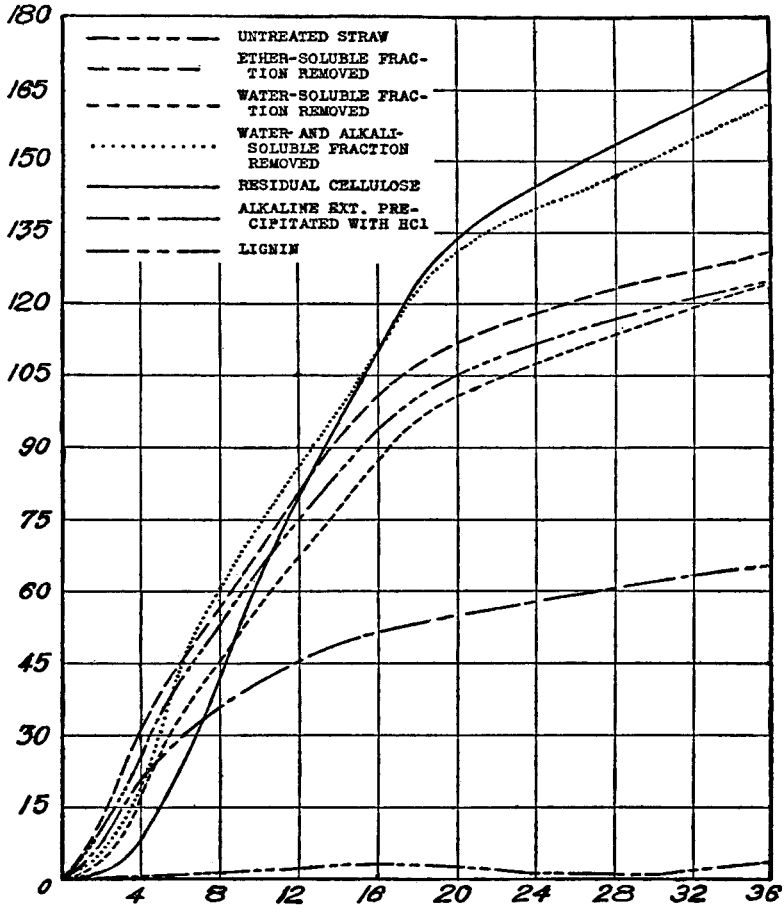

FIG. 1.—COURSE OF DECOMPOSITION OF CEREAL STRAW AND ITS VARIOUS FRACTIONS (16)

An examination of the lignin fraction in plant material will amply bear out the above statement. It has been shown (17) recently that the lignin content increases with advancing maturity of the plant. The chemical nature of the lignin and its relationship to the various other plant con-

stituents also change when the plant matures. Finally, whereas lignin in mature plant materials is almost resistant to decomposition, lignin in young plants may be readily attacked by microorganisms.

The hemicellulose fraction of the nitrogen-free extract consists not only of polymers of hexoses, pentoses and hexose carboxylic acids, but also of mixtures of these. Preparations of hemicelluloses from different plants or even from the same plant at different stages of growth will vary in chemical composition and, therefore, in the nature and extent of their decomposition. Certain investigators (7) differentiated between free and combined hemicelluloses, on the basis of their solubility in 4 per cent sodium hydroxide solution, the first group being soluble in the cold and the second only on heating. It is largely because of this variability in nature and in concentration that one finds in the literature (12) statements to the fact that hemicelluloses are readily decomposed by a great variety of organisms, whereas others find that certain hemicellulose preparations, like agar-agar, are among the most resistant constituents of plant materials.

In order to illustrate the lack of correlation between the composition of a plant product, as determined by the methods of foodstuff analysis, and the degree of its digestibility by animals or decomposition by microorganisms, it is sufficient to point to peat materials. In spite of their high con-

TABLE 2.—*Influence of age of rye upon its composition*

(On per cent basis of dry material.)

AGE OF PLANT	I 10–14 INCHES	II JUST BEFORE HEAD FORMATION	III JUST BEFORE BLOOM (STEMS AND LEAVES)	IV MATURE PLANTS (STEMS AND LEAVES)
Ether-soluble portion	2.60	2.60	1.70	1.26
Cold water-soluble portion	34.24	22.74	18.16	9.90
Hemicelluloses*	16.60	21.18	22.71	22.90
Cellulose	18.06	26.95	30.59	36.29
Lignin	9.90	11.80	18.00	19.80
Ash	7.66	5.90	4.90	3.90
Total nitrogen	2.50	1.76	1.01	0.24

* Only pentosans determined.

tent of nitrogen-free extract, especially in the case of high-moor or sphagnum peats, they are highly resistant to microbial decomposition (15), as well as to digestion by animals (3), (9).

Knowledge of the chemistry of lignins is still insufficient to enable one

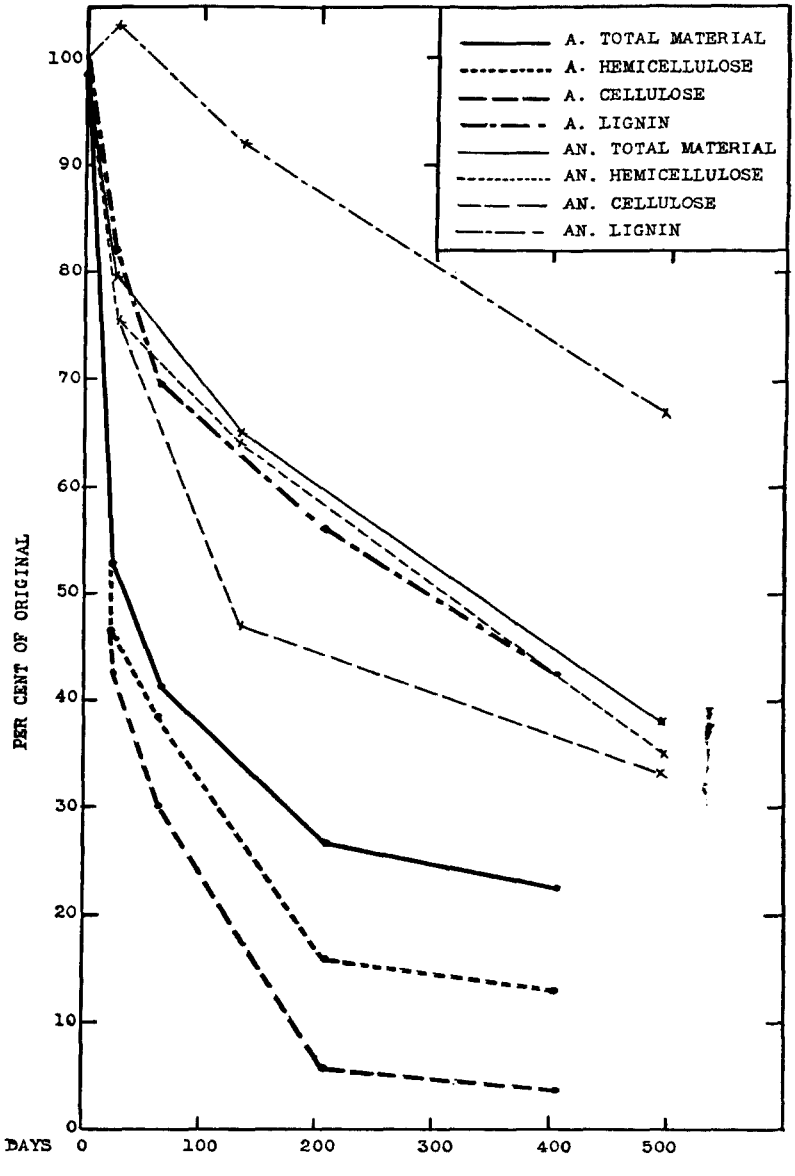

FIG. 2.—COMPARISON OF ANAEROBIC (AN.) AND AEROBIC (A.) DECOMPOSITION OF CORN (10)

to connect differences in their digestibility with their chemical nature. In the case of some plant materials, as in young rye plants or in leguminous plants, the lignins are more or less readily decomposed, less so than the hemicelluloses and cellulose. However, in other materials, such as mature cereal straw or wood, the lignins are much more resistant to decomposition (13), (11). At higher temperatures, as in the case of hot composts, lignin may be attacked more readily than at lower temperatures. Not only the temperature, but the oxygen tension and other environmental factors also influence the rate of lignin decomposition (10).

The existence of a definite interrelationship in the plant materials between the hemicelluloses and the lignin has been suggested. According to some investigators (6), hemicelluloses and lignin form in the plant material a combination, the solution of the hemicelluloses depending upon the rupture of the linkage between it and the lignin. An attempt has been made (8) to correlate the degree of decomposition of plant materials with the abundance of pentosans and lignins. This conception was not accepted however. Plant materials were found to decompose at a rate that is roughly in inverse proportion to their lignin content, the ratio of decomposable material being the sum of hemicelluloses and cellulose to lignin (5).

Lignins are not only more resistant to decomposition by microorganisms than carbohydrates, but have a definite retarding effect upon microbial decomposition of proteins (14). This phenomenon was explained by a possible chemical combination between the two compounds. Whether this effect is due to direct chemical interaction or to a mere physical protection of the protein by the lignin remains to be established. Because of the greater resistance of the lignins to decomposition and because of their specific protective effect upon proteins, they, as well as the proteins, contribute largely to the formation and accumulation of soil humus.

These facts definitely emphasize the importance of a knowledge of the chemical composition of those compounds that make up the nitrogen-free extract fraction of plant materials. Any generalizations based upon the transformation of only one of the constituents of this fraction may be hitting far from the mark because of the complex and varying chemical composition of this fraction.

Tables 1–3 and Figures 1–2 taken from some of the published results of this laboratory will help to elucidate some of the preceding statements.

Summary.—The contribution of a plant material to the formation of soil humus depends largely upon three factors: (1) the chemical composition of the material; (2) conditions of its decomposition (moisture, aeration, reaction); and (3) presence of supplementary nutrient elements and specific microorganisms. In the case of properly controlled composts and normal soils, the first factor is the one that controls chiefly the rate of decomposition of the material.

A knowledge of the chemical nature of the nitrogen-free extract is essential in order to determine its contribution to the soil humus. Because

TABLE 3.—*Chemical changes produced in decomposition of horse manure*
(On basis of 1000 lbs. fresh manure, containing 70.2% moisture.)

CHEMICAL CONSTITUENTS	TIME OF ANALYSIS					
	AT START		27 DAYS		63 DAYS	
	RELATIVE CONCENTRATION	TOTAL WEIGHT	RELATIVE CONCENTRATION	TOTAL WEIGHT	RELATIVE CONCENTRATION	TOTAL WEIGHT
	per cent	*pounds*	*per cent*	*pounds*	*per cent*	*pounds*
Ether-soluble fraction	2.87	8.6	1.36	3.1	1.10	2.5
Cold water-soluble organic matter	3.64	10.8	4.72	10.6	3.31	7.4
Hot water-soluble organic matter	1.48	4.4	5.00	11.2	3.89	8.7
Hemicelluloses	17.78	53.0	15.40	34.5	13.02	29.2
Cellulose	28.35	84.5	26.32	59.0	15.59	35.0
Lignin	15.64	46.6	20.44	45.8	27.02	60.6
Water-insoluble protein	7.81	23.3	11.56	25.9	13.13	29.5
Total nitrogen	1.44	4.3	1.92	4.3	2.35	5.3
Ash content	13.48	40.2	14.50	32.5	15.49	34.8

of the indefinite nature of the chemical constituents comprising this fraction, it is impossible to state beforehand how rapidly and how completely it will decompose and how much it will contribute to humus formation. Broadly speaking, the starches and sugars, followed by certain hemicelluloses, will tend to decompose rapidly, giving rise to extensive microbial cell substance. Some of the hemicelluloses and the lignins will tend to accumulate. The cell substance synthesized by the various soil microorganisms will vary in chemical composition, depending upon the nature of the microbiological population and upon the conditions of decomposition of the various organic compounds. This cell substance will not remain as such but will undergo in the soil and in the compost a series of changes, both chemical and microbiological, giving rise to more resistant substances. These, together with the lignins and some of the resistant hemicelluloses, will form the major contribution of the nitrogen-free extract to the soil humus.

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NITROGEN-FREE EXTRACT FROM THE PLANT-PHYSIOLOGICAL VIEWPOINT

By F. E. DENNY (Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.)

Those who, years ago, developed and secured the adoption of the nitrogen-free extract system of analysis for feeding stuffs were, of course, under no obligation to bear in mind the special requirements of the plant physiologists—if, indeed, they knew that the tribe existed—or cared.

It was up to the plant physiologists to make use of the information as best they could. By this procedure the information is received in packages each containing somewhat similar but by no means identical items. Experience shows that there are at least two of these packages that in most cases need to be untied so that the contents can be reassorted, or as the modern parlance is, so that the values can be "broken down." One of these is the fraction called N×6.25, and the other is the subject of this symposium.

From the viewpoint of plant physiology, the difficulty with the computed value for the nitrogen-free extract is not so much that its true value is uncertain on account of the accumulation of errors in the various determinations, but that the value, as finally stated, represents a group of substances about none of which does the computation give any information. It seems that it might be more appropriately called merely the "undetermined fraction."

No matter how accurately its value may be known, it still represents a mixture, and progress in plant physiology seems to be dependent upon the

recognition of the presence of definite chemical constituents, and the successful estimation of changes in each separately, both as to time of change and the position finally taken by the substance within the plant body.

Not only does this system of analysis combine into one estimate a large number of not very closely related substances, but, unfortunately, this group contains so many of the tissue constituents that have special interest, which are believed to be functioning actively in metabolism, or at any rate, components regarding which much information is being sought and found at the present time.

For example, some of the constituents that may be found lumped together in the computed value called the nitrogen-free extract are: starch, sugar, organic acids, some of the glucosides, hemicelluloses, pectins, various non-sugar reducing substances, glucosans, fructosans, toxins, etc. It can readily be seen that a value representing the combined weight of these, and perhaps many other even unknown constituents, would add practically nothing to our knowledge of the physiological activity of the tissue that contained them.

Starch.—Since in most species of plants, starch constitutes the basic storage material (the savings account, as it were) out of which funds are obtained for operating the entire system, it is quite essential for our purpose that this item be estimated *separately* from all other compounds. Plant physiologists are very grateful to the analytical chemists for the excellent methods of estimation that they have developed. Yet, in many cases, these methods are insufficient for the reason that (of all reasons!) there is too much starch. The fact is that in many physiological processes the amount of sugar involved is so small that the accompanying change in starch is about equal to or less than the error of the determination, good as it is. It is not possible in such cases to set up a system of accounting that will give a complete picture of the details of the transaction.

At the other extreme, there are tissues containing very little starch, or tissues from which the starch gradually disappears with time. This small amount of starch is often accompanied by large amounts of related polysaccharides, and in many cases it is necessary to determine the starch accurately in the presence of this mixture. Much progress analytically has been made in this field, and new procedures are being proposed and tested. Occasionally, however, an author of one of these new methods does not try the method out on a tissue that *contains no starch.* The question is, can he go through his procedure and get a zero reading for starch when starch is absent? This is a critical point and has not yet been met in all cases.

It is clear that if plant physiologists are fussy about such points they obviously cannot be much interested in the computed value of the nitrogen-free extract in which the starch is merely included with many other constituents in a single figure.

Sugar.—This constituent of the nitrogen-free extract must in nearly all physiological studies be separately estimated. The methods for this purpose are so varied and so generally satisfactory that no difficulty is usually met in obtaining a value which is acceptable to all concerned. The principal difficulty is usually in finding out what the meaning of the value is after it has been obtained.

It seemed obvious at first to almost everyone that since sugar is the substrate for energy release and is the basic substance for the construction of various constituents of the tissue, a good sugar analysis should allow some definite conclusions. It is not so easy as that. It is probably true that sugar is used up in respiration, but the view previously advanced and still held, at least by some investigators, that the sugar concentration regulates the rate of respiration is simply not true. Sugar is not always, or perhaps ever, the sole substrate for respiration. Miller,[1] shows results in which the concentration of sugar and rate of respiration at a certain stage are inversely correlated.

Organic Acids.—Other components of the nitrogen-free extract which for our purpose must be dealt with separately are the organic acids. They may amount to 10 per cent of the dry weight of some tissues, or in other cases, 30 per cent of the organic content. They change in amount from morning to night and reverse this procedure from night to morning. Recent investigations by Pucher, Wakeman, and Vickery[2] show the interesting fact that in this respect the behavior may be and is just the opposite in two different species of plants, that is, in one species the acid increases in the day and decreases at night, in the other species it decreases during the day and increases at night. They also show[3] that the short-cut methods previously used in which conclusions regarding the organic acid content of a tissue were reached by merely determining the *p*H and the titratable acid are misleading, and they emphasize that to understand the situation correctly the content of inorganic ions, organic bases, and the proportion of the various individual acids themselves must be known.

An interesting feature of organic acid metabolism was brought out by my co-worker, J. D. Guthrie. In daylight certain types of plants become less acid and they become acidified again on being returned to darkness. Guthrie[4] found that after the plants had remained in darkness and had reached an equilibrium the loss of acid that occurred in the light could be produced in darkness by treating the plants with vapors of ethylene chlorhydrin. Why should ethylene chlorhydrin act as a substitute for light in such a case? Chlorhydrin decreases the acid by an effect upon the respiration. Does light produce its effect in a similar way?

This organic acid phase of plant metabolism is becoming increasingly

[1] *Contrib. Boyce Thompson Inst.*, 5, 213 (1933).
[2] *J. Biol. Chem.*, 126, 43 (1938).
[3] *Plant Physiol.*, 14, 333 (1939).
[4] *Contrib. Boyce Thompson Inst.*, 8, 283 (1936).

interesting and is yielding important advances in such fields as respiration, photosynthesis, and nitrogen metabolism. But this progress was not made by letting the acids stay in the nitrogen-free extract, but rather by rescuing them from it, and dealing with them individually as separate and distinct components.

Glucosides.—Another series of substances likely to be included in the nitrogen-free extract but deserving of separate estimation are the glucosides. Some of them, it is true, contain nitrogen and may be included not in this fraction but under the heading of N×6.25. But they would be equally out of place if lumped in either category. From the viewpoint of plant physiology they may need to be analyzed for separately. In many or most cases the task will be found difficult and probably one for the chemist rather than the plant physiologist. These constituents are grouped under a single name, glucoside, but their physiological effects are quite variable. One may be a poison, yet another is thought to be indispensable for animal life. One may be present in amounts as high as 15–20 per cent of the dry weight without any conclusive evidence having been produced that it is playing any critical part in plant metabolism, and another, although present in extremely small amounts, is so active that it produces an effect in a certain system at a dilution of one part in 250 trillion. The diversity in character of these glucosides is so great that it must be doubted whether they have been classified properly in the chemical system, emphasizing as it does the sugar constituent rather than the aglucon from which perhaps the member inherits its principal traits.

In any event the presence of a glucoside in any tissue may be suspected, and any attempt to understand the physiology of the plant would require that its value not be allowed to remain in the sum of the substances computed as nitrogen-free extract, but that it be isolated, identified, and dealt with separately, as accurately as necessary, or as permitted by the knowledge and skill of the operator.

L. P. Miller has recently opened up a new and interesting phase of the study of plant glucosides. The germination of potato tubers and gladiolus bulbs is hastened by treatment with ethylene chlorhydrin. Miller measured the amount of chemical that went into the tissue, the amount that came off again later as a vapor, and the amount that could be recovered unchanged from the tissue. The difference, or the amount still unaccounted for, turned out to be most of all of the chemical that was originally applied. Of the chemical that gets into the tissue and stays a little while, long enough for the tissue to act upon it, nearly all is captured by the tissue and is transformed by it into something which is no longer ethylene chlorhydrin. Miller[5] found that the tissue (either potato or

[5] *Contrib. Boyce Thompson Inst.*, 9, 425 (1938).

gladiolus) synthesized a glucoside in which ethylene chlorhydrin was the aglucon. The tetraacetates from the natural product and that produced by artificial synthesis were identical. Thus, it is believed that for the first time higher plants have been detected in the process of producing a glucoside from an added aglucon which was not itself a naturally occurring constituent. Miller later (unpublished) obtained glucosides from tissues other than these and with at least one chemical other than ethylene chlorhydrin.

Hemicellulose.—This is another constituent of the nitrogen-free extract that must be estimated separately in order to contribute to our understanding of its role in the life of the plant. Even when this is done, the place it holds in physiology is not at all clear.

Hemicellulose may amount to 20–30 per cent of the dry weight of certain tissues, and according to some authors the amount undergoes a progressive change according to the seasons of the year, and in accordance with the stage of development of the plant. If this is so, this fraction must be playing an important role in metabolism. But Winkler and Williams[6] claim that with grape vines this so-called change is only an apparent one, that changes in the percentage of hemicellulose are in reality mainly due to changes in other constituents that contribute to the total weight, and so when the percentage composition is computed the hemicellulose values are falsely reflected in the resulting ratio. On this basis previous investigators may have proved merely that the value of a ratio is influenced not only by the numerator but also by the denominator.

And so, once more, it seems to be easier to get figures than to find out what they mean.

Conclusion.—The basic object of chemical plant physiology may be considered to be the preparation of a balance sheet by which an accounting as complete as possible may be made for income, outgo, and transformation of materials. For such a problem the computed figure obtained for the nitrogen-free extract is of little value. With it is obtained only a single entry to represent the total of a variable but always large number of constituents, many of which certainly are playing important roles in metabolism (and as for the others we have our suspicions). For studies in plant physiology the value for nitrogen-free extract would have to be "broken down," so that the various constituents present may be known individually with as much accuracy for each as may be obtainable.

And so, it appears that when the feeding-stuff chemist has completed his analysis, and has settled back with a sigh of satisfaction with a job well done, the troubles of the plant physiologist have just begun.

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[6] *Plant Physiol.*, 13, 381 (1938).

NITROGEN-FREE EXTRACT IN ANIMAL NUTRITION

By L. A. MAYNARD (Laboratory of Animal Nutrition, Cornell University, Ithaca, N. Y.)

As determined by the customary procedure for the analysis of feeding stuffs nitrogen-free extract is the largest component of the rations of animals, representing 40–70 per cent of the total dry matter. It serves as a source of energy for body processes and for the deposition of fat. But it is not the total amount of nitrogen-free extract present nor its gross calorie content that is most important nutritionally. Rather the nutritionist is primarily interested in that fraction of the gross energy which is available and useful to the body. This availability is governed by digestibility and other metabolic factors, which in turn are directly related to the chemical nature of the nitrogen-free extract. Thus the nutritionist has a vital interest in knowing more about the chemistry of this feed constituent.

As previously discussed in this symposium, nitrogen-free extract contains starch, hemicellulose, lignin, pectin, and related substances. Starch as such is almost entirely digested by the animal under the action of intestinal enzymes. On the other hand, there are no enzymes secreted by mammalian tissues which will digest cellulose or lignin, and this appears to be true also for at least most of the compounds that make up the group called hemicellulose. Several investigators have reported failure to find a pectin- or an inulin-digesting enzyme in animal tissues.

While digestive enzymes are lacking for these various higher carbohydrates, the latter are nevertheless subject to breakdown in the animal body under the action of microorganisms, notably bacteria and infusoria, which inhabit certain portions of the alimentary canal. This microbial action occurs to a substantial degree only in those species which have a digestive tract suitable for the purpose, namely, the herbivora. The action takes place to the fullest extent in the ruminant, but it also occurs to a large degree in the ceca of non-ruminants such as the horse and the rabbit. Since there are marked differences among the different higher carbohydrates in their susceptibility to attack by microorganisms one would expect differences in the extent to which they are broken down by the organisms in the digestive tract. Such is the case.

Indirect evidence that microbial digestion of a given constituent can take place is obtained by the isolation from the digestive tract of organisms which break down the substance in question. Direct evidence is furnished by a digestion trial. This procedure has been of very limited usefulness thus far as regards certain of the constituents of the nitrogen-free extract, because of a lack of reliable, quantitative chemical methods, particularly as applied to feedstuffs and mixtures of them.

The low degree of digestibility of lignin has long been recognized. In

our laboratory we have recently given some attention to the digestibility of this substance by different species, in comparison to that of cellulose. Some of the data obtained have been published by Crampton and Maynard.[1]

As an illustration some unpublished data by Loosli and Maynard are presented in Table 1, giving results obtained with different species fed

TABLE 1.—*Digestibility of cellulose and lignin in alfalfa hay*

SPECIES	LEVEL IN RATION		DIGESTIBILITY	
	CELLULOSE	LIGNIN	CELLULOSE	LIGNIN
	per cent	*per cent*	*per cent*	*per cent*
Rabbit	32	14	28	0
Guinea pig	32	14	41	5
Lamb	32	14	58	28

alfalfa hay. These data show that in the rabbit and guinea pig the digestion of lignin is practically nil. The coefficient for lambs, which is larger than other data we have obtained with ruminants, indicates a substantial degree of digestibility. Even so the figure is less than half that for cellulose digestion.

Similar data have been obtained in other trials. The chemical methods used have been described by Crampton and Maynard.[1] It is recognized that these methods have limitations. Even so, they have been found distinctly useful in studying the utilization of the higher carbohydrates of forage crops by animals, as is indicated by data discussed later.

The digestion data indicate clearly that the lignin component of the nitrogen-free extract is of very low digestibility compared to cellulose, which is found in the crude fiber fraction as determined in the conventional method of feed analysis. Hemicellulose appears to have a somewhat higher digestibility than cellulose, but generalizations are dangerous here because of the variety of the compounds included in this group and the uncertainties involved in the methods used. The digestibility of pentosans by sheep, as determined by the furfuraldehyde method, has been reported to vary from 55 to 95 per cent in different feeds. Bacteria that will break down pectin have been isolated from the digestive tract of herbivora, and its nearly complete digestion for various species has been noted.

It should be emphasized, however, that digestion as customarily measured merely denotes the disappearance of the compound in question in passing through the tract, or perhaps its conversion into fecal constituents not accounted for by the method used for the original substance. While the digestion product of starch and its value to the body are well understood, this is much less true for the higher carbohydrates. It is

[1] *J. Nutrition*, 15, 383 (1938).

recognized that the products of the microbial digestion of cellulose and related compounds are largely organic acids, some of which appear to be of no use to the body. Some energy is also lost in gaseous form. These facts have proved difficult to reconcile with the early findings of Kellner that digested cellulose and pentosans have as great a fattening value (net energy value) as starch. Kellner, Tollens, and others recognized that starch itself might be subject to methane fermentation and this was offered as a basis for considering the digested crude fiber and digested nitrogen-free extract to have the same nutritive value. Though this explanation does not seem adequate, the early work of Kellner has not been disproved. Thus, in our present feeding standards based on total digestible nutrients, the digested constituents of both the nitrogen-free extract and crude fiber fractions are all given the same value for energy purposes. Much more study is needed here by the physiologist; but in many cases the development of better chemical methods must come first, in order to follow more accurately the digestion and metabolism of the compounds concerned.

Lignin makes up a substantial percentage of the constituents of many feeds, particularly forage crops. Its significance is heightened by the fact that, in addition to being of low digestibility, its presence tends to lower the digestibility of the other constituents, apparently by protecting them from the action of the digestive juices and other agencies. Further, it is recognized that the lignin content of forage crops is subject to marked variation according to stage of maturity and to climate and other cultural factors. It is evident that the nature of the lignin itself and its digestibility are influenced by some of these factors. These facts have an important practical bearing on cultural practices, on the time crops should be cut for hay and silage, and on the nutritive value of pasture grass. As an example, some data obtained by Norman[2] showing the effect of stage of growth on the chemical composition of rye grass may be cited. Over the period studied the protein content decreased 50 per cent, the cellulose content doubled and, most significant, the lignin content increased fivefold.

Crampton[3] has combined chemical studies with animal experiments to show the effect of lignin content on digestibility and growth. Using pasture herbage clipped periodically throughout the season, he obtained the lowest digestibility and the poorest growth with the sample highest in lignin.

I have given these detailed figures regarding lignin because they provide important evidence that, from the standpoint of animal nutrition, the present procedure of partitioning the carbohydrates of feeds into crude fiber and nitrogen-free extract is not very satisfactory as a means of separating the less useful carbohydrates from the more useful ones, as regards digestibility and net energy value. It has the advantage of sim-

[2] *Agr. Prog.*, 14, 141 (1937).
[3] *Sci. Agr.*, 19, 6, 345 (1939).

plicity for routine analysis and for control work, and clearly it has served a useful purpose. But the recently acquired knowledge of the constituents of the two fractions and of their digestibility has served to emphasize the limitations of the procedure. That digestibility may not follow the classification at all closely, particularly forage crops, is indicated by the data compiled by Crampton and Maynard[1] from published digestion coefficients.

As has been pointed out by Dr. Browne in this symposium, Henneberg and the later German workers recognized that nitrogen-free extract contained some indigestible material, notably lignin, and that the crude fiber fraction was in part digestible. Dr. Browne has referred to the recognition of "compensation" factors whereby, as indicated by the table from Tollens' work, the partition did appear to measure the relative amounts of digestible and undigestible material. A study of modern digestion trials, however, makes it evident that this "compensation" does not work out satisfactorily in many cases, as is illustrated in Table 2. Some of these

TABLE 2.—*Application of "compensation" to modern digestion data*

FEED	SPECIES	DIFFERENCE, AS PER CENT OF N.F.E., BETWEEN N.F.E. AND SUM OF DIGESTED N.F.E. AND DIGESTED CRUDE FIBER*	SOURCE OF DATA
Dried grass	Cow	11.9	Vermont Experiment Station
Green grass	Cow	11.1	
Alfalfa silage	Cow	5.2	
Timothy silage	Cow	19.1	
Soybean silage	Cow	− 6.6	
Oat feed	Horse	−26	Wisconsin Experiment Station
Oat feed	Cow	−22	
Oat feed	Sheep	−23	
Oat feed	Hog	−40	
Alfalfa hay, very early	Cow and sheep	− 0.5	Morrison's Tables
Alfalfa hay, stemmy	Cow and sheep	16.0	
Alfalfa, green, immature	Cow and sheep	− 6.8	
Pasture grasses and clover	Cow and sheep	16.4	
Beet pulp, dried	Cow and sheep	10.4	
Corn, dent	Cow and sheep	− 4.2	
Distillers' grains, dried	Cow and sheep	2.3	
Linseed meal, o.p.	Cow and sheep	− 5.0	

* Where the sum of the digested N.F.E. and digested crude fiber was smaller than the total N.F.E. the difference is indicated by a minus sign.

data were chosen for the specific purpose of showing extreme cases, but they show that "compensation" cannot be relied upon to make the analytical data for nitrogen-free extract and crude fiber reliable measures of digestibility.

In studies of the digestibility of animal products by mink further evidence was obtained by Loosli and Smith in this laboratory of the limitations of the analytical procedures. The application of the crude fiber method to frozen horse muscle meat has given values ranging from 2 to 3 per cent. Certainly the substance here determined can not be a carbohydrate. A fish meal fed in a digestion trial was found to contain 0.9 per cent of nitrogen-free extract. This figure was not surprising considering that it is determined by difference. But when the feces obtained from the feeding of a given quantity of fish meal was analyzed the output was found to contain 10 times as much nitrogen-free extract as was consumed. This observation was confirmed with other samples of meal and by other animals. It is chemically correct by the difference method, but physiologically impossible. Studies are now in progress to ascertain what fecal constituent was erroneously determined as nitrogen-free extract by the difference procedure. Until this question is answered one cannot tell whether the digestion coefficients obtained for any of the other nutrients are reliable.

It seems clear that, at least from the standpoint of research, more specific methods for the determination of the higher carbohydrates are needed. This is particularly true for the rations of herbivorous animals. A step in this direction has been made in the study by Crampton and Maynard.[1] A modified procedure for feeding stuff analysis was developed, involving the determination of cellulose and lignin. A comparison of the results thus obtained with those of the conventional method shows that the modified procedure is more specific and informative in that it determines the least digestible carbohydrate constituent, lignin, and also determines cellulose, the principal constituent of the crude fiber fraction, and results in a smaller undetermined fraction ("other carbohydrates") to be arrived at by difference. The usefulness of the procedure has been further demonstrated by Crampton in connection with feeding trials.

This modified scheme is not considered a finished product but merely a promising basis for further work. Further testing of the methods for cellulose and lignin is required before much improvement can be expected. Though the scheme results in a smaller fraction to be determined by difference the magnitude of this residue clearly suggests the need for its more definite characterization. Significant differences in its nutritive value may well occur according to its make-up. The importance of further chemical studies is clearly indicated.

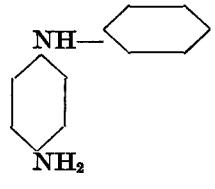
From the standpoint of routine feed analysis and of control work any procedure designed to be substituted for the present partition into nitrogen-free extract and crude fiber must not be too complicated or time consuming. These limitations do not apply to the broader and more fundamental objectives of supplying better tools for research in the field of animal nutrition.

The major role which the higher carbohydrates play as energy foods, especially for herbivora, and the fact that feeds which appear to have a similar composition as measured by the present conventional method are frequently found to vary markedly in actual feeding values, emphasize the importance of the development and use of more exact chemical and biological methods for the constituents of nitrogen-free extract. No group of nutrients that makes up 40 per cent of the total should be determined by difference.

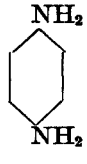
SEPARATION AND DETERMINATION OF 4-AMINODIPHENYLAMINE

By I. S. SHUPE (Cosmetic Division,* U. S. Food and Drug Administration, Baltimore, Md.)

The monohydrochloride of 4-aminodiphenylamine is a common constituent of certain hair dyes and forms a dark blue solution on oxidation. The relationship of the free base to paraphenylenediamine is readily seen by a comparison of the structural formulas of the two compounds:



4-aminodiphenylamine or phenyl-paraphenylenediamine



paraphenylenediamine

4-Aminodiphenylamine is relatively insoluble in water, slightly soluble in petroleum ether, and very soluble in chloroform, ether, and benzene.

Although not extremely soluble in petroleum ether, 4-aminodiphenylamine may be extracted quantitatively with this solvent. In this respect it differs from many related amino compounds, such as paraphenylenediamine, metaphenylenediamine, paratoluenediamine, 2,4-aminoanisole, ortho- and para-aminophenols.

The following method of separation and determination of 4-aminodiphenylamine is based on an extraction with petroleum ether:

METHOD

Transfer a portion of sample, calculated to yield not more than 0.1 gram of 4-aminodiphenylamine, to a separatory funnel. (Dissolve a solid material in water or dilute HCl. Heat if necessary for solution.) Make alkaline with excess of powdered $NaHCO_3$. Extract with one 50 cc. and four 20 cc. portions of petroleum ether. Wash the combined petroleum ether extracts of 20 cc. of water, and wash this water with

* D. Dahle, in charge.

one 20 cc. portion of petroleum ether, adding this portion to the other petroleum ether extracts. Filter the ether extracts through a pledget of cotton into a tared dish. Evaporate on a steam bath, using a current of CO_2 to aid evaporation. Permit the last few cc. of ether to evaporate without heat to avoid loss by spattering. Dry in a desiccator to constant weight or at 100° C. for 30 minutes and weigh. The residue is 4-aminodiphenylamine.

Acetyl Derivative

Dissolve a weighed portion of the extracted base in 5 cc. of 1 % HCl. Add 0.5 cc. of acetic anhydride for each 0.1 gram of base, then about 1 gram of $NaHCO_3$. Place on the steam bath for a few minutes until excess acetic anhydride is dissipated. Add 5 cc. of water and let stand in the cold for 1 hour. Filter onto a tared Gooch crucible, using about 25 cc. of water for transferring and washing the precipitate. Dry at 100° C. for 1 hour and weigh. The acetyl derivative is slightly soluble in water, and with the above experimental conditions a correction of 0.7 mg. should be added to the weight of residue.

Acetyl derivative $\times 0.9755$ = 4-aminodiphenylamine · HCl.

Acetyl derivative $\times 0.8142$ = 4-aminodiphenylamine.

The acetyl derivative recystallized from alcohol and water melts at 162–3° C. Deshusses,[1] using a different method for preparing the acetyl derivative, reports the melting point as 165° C.

Benzene-Sulfonyl Derivative

Dissolve a weighed portion of the extracted base in 5 cc. of alcohol, and add 0.5 cc. of benzene-sulfonyl chloride for every 0.1 gram of base and 5 cc. of 25% sodium acetate. Heat on a steam bath for 30 minutes, dilute with 30 cc. of water, and let stand for 1 hour with occasional stirring to aid crystallization. Filter onto a tared Gooch crucible, using cold water for transferring and washing the precipitate. Dry at 100° C. for 1 hour and weigh the benzene-sulfonyl-4-aminodiphenylamine.

Benzene-sulfonyl derivative $\times 0.6804$ = 4-aminodiphenylamine · HCl.

Benzene-sulfonyl derivative $\times 0.5679$ = 4-aminodiphenylamine.

The benzene-sulfonyl derivative, recrystallized from alcohol and water, melts at 138–9° C.

Recoveries of 4-aminodiphenylamine by petroleum ether extraction of the pure salt and of mixtures of amino compounds are given in Tables 1, 2, and 3.

TABLE 1.—*Recoveries by extraction*

EXPERIMENT NUMBER	WEIGHT OF 4-AMINODIPHENYLAMINE HYDROCHLORIDE	SOLVENT USED	WEIGHT OF BASE RECOVERED	RECOVERY
	mg.		*mg.*	*per cent*
1	116.0	$CHCl_3$	96.8	100.0
2	99.2	Pet. Ether	82.2	99.3
3	49.6	Pet. Ether	41.3	99.8
4*	49.6	Pet. Ether	43.0	103.8

* NaOH used instead of $NaHCO_3$ to make the solution alkaline before extraction.

[1] *Mitt. Lebensm. Hyg.*, 30, 1/2, 10 (1939).

In experiments 3 and 4 the following quantities of other amino compounds were added to the 4-aminodiphenylamine:

	mg.
paraphenylenediamine	300
metaphenylenediamine	300
2, 5-diaminotoluene	300
o-aminophenol	60
p-aminophenol	60
2,4-diaminoanisole	30
p-methylaminophenol	100

TABLE 2.—*Recoveries based on the acetyl derivative*

EXPERIMENT NUMBER	WEIGHT OF 4-AMINO-DIPHENYLAMINE	WEIGHT OF ACETYL DERIVATIVE	RECOVERY*	
			UNCORRECTED	CORRECTED FOR SOLUBILITY OF DERIVATIVE
	mg.	*mg.*	*per cent*	*per cent*
1	20.7	24.7	97.1	100.0
2	41.4	49.8	97.8	99.3
3	82.8	101.0	99.3	100.0

* Recovery based on monoacetyl-4-aminodiphenylamine ($C_{12}H_{11}N_2 \cdot CH_3CO$). Mol. Wt. 226.13.

TABLE 3.—*Recoveries based on the benzene-sulfonyl derivative*

EXPERIMENT NUMBER	WEIGHT OF 4-AMINO-DIPHENYLAMINE	WEIGHT OF BENZENE-SULFONYL DERIVATIVE	RECOVERY*
	mg.	*mg.*	*per cent*
1	20.7	36.3	99.5
2	41.4	72.8	99.8

* Recovery based on monobenzenesulfonyl-4-aminodiphenylamine ($C_{12}H_{11}N_2 \cdot C_6H_5SO_2$). Mol. Wt. 324.21.

DISCUSSION

Griebel and Weisz[2] give color tests for the identification of 4-aminodiphenylamine and make a partial separation from paraphenylenediamine and paratoluenediamine by repeated washing with water of an ether solution of the bases. The latter two bases, being more soluble in water, are gradually removed.

Deshusses[1] for identification purposes describes a partial separation of the 4-amino compound from the diamines by fractional crystallization of the acetyl derivatives.

The free base crystallized from petroleum ether has a characteristic needle-like structure and melts at 66–7° C. This material can be dried at 100° C. without loss in weight, after which upon crystallization it has a melting point of 73–4° C. A chloroform extract of the base sublimed in

[2] *Z. Untersuch. Lebensm.*, **70**, 61 (1935).

vacuum melts at 74–5° C. The petroleum ether extract apparently does not sublime but upon vacuum distillation at a low temperature gives a product melting at 66–7° C.

Apparently 4-aminodiphenylamine can be obtained in two allotropic modifications, one melting at 66–7° C. and the other at 74–5° C.

Both of these products give the same acetyl and benzene-sulfonyl derivatives.

SUMMARY

A method is suggested for the separation, identification, and quantitative determination of 4-aminodiphenylamine in the presence of various related amino compounds. Acetyl- and benzene-sulfonyl derivatives have been studied. Quantitative data on recoveries are given.

ESTIMATION OF IODINE IN SOILS, PLANT MATERIAL, AND WATERS

By G. S. FRAPS and J. F. FUDGE (Division of Chemistry, Texas Agricultural Experiment Station, College Station, Texas)

The Association of Official Agriculture Chemists has adopted tentative methods for the estimation of iodine in soils,[1] brine,[2] and plant material,[3] Difficulties encountered by the writers in connection with the estimation of iodine in soils by the fusion method have been discussed.[4] Occasionally trouble has also been experienced when iodine was estimated by the use of sodium nitrite in the solution of the salts extracted by ethyl alcohol from the original ash or residue of plant material and waters. Most of these difficulties seemed to be due either to loss of iodine or to interference of other substances with the liberation of iodine. This paper presents and discusses the method that was finally adopted for the estimation of iodine in soils, plant material, and waters.

The method is a colorimetric modification of the method proposed and later modified by Trevorrow and Fashena.[5] It consists of a preliminary burning of plant material, then an oxidation of organic matter by chromic acid, during which the iodine is oxidized to iodic acid, reduction of the iodic acid to iodine with phosphorous acid, and distillation of the iodine in a special all-glass apparatus. The iodine in the distillate may be liberated by means of nitrous acid and the color read directly, or it may be oxidized to iodic acid by treatment with bromine, the iodine liberated by a dilute solution of potassium iodide, the iodine extracted with carbon

[1] *Methods of Analysis*, A.O.A.C., 1935, 8.
[2] *Ibid.*, 528.
[3] *Ibid.*, 133.
[4] *This Journal*, 18, 314 (1935).
[5] *J. Biol. Chem.*, 110, 29 (1935); 114, 351 (1936).

tetrachloride, and the color compared with a standard. The latter procedure is preferred. The details of the method follow:

IODINE IN SOILS, PLANT MATERIAL, AND WATERS

REAGENTS AND APPARATUS

(a) *Water.*—Redistil distilled water from a K_2CO_3 solution in a glass apparatus (necessary only if blank shows presence of interfering substances in water).

(b) *Potassium dichromate.*—Recrystallize twice from distilled water, wash with purified alcohol, and dry in an oven at 110° C.

(c) *Chromic acid-sulfuric acid mixture.*—Dissolve 3 grams of K_2CrO_4 and 5 cc. of water in 95 cc. of concentrated H_2SO_4.

(d) *Cerous sulfate.*—Wash a C. P. grade three times with purified alcohol.

(e) *Phosphorous acid.*—The acid can be purified by steam distillation until the distillate is free of chlorides, but purification is not necessary if the blank is zero. Keep the volume of the acid constant by heat from a second burner.

(f) *Sodium hydroxide solution.*—0.02 *N*.

(g) *Carbon tetrachloride.*—In most cases regular C. P. CCl_4 is satisfactory, as it contains no interfering substances and gives no blank for iodine. When necessary it may be purified by treating 500 cc. with 50 cc. of bromine water and allowing to stand several hours in the sunlight. Add an excess of NaOH and let stand overnight. Wash the solution three times with water, separating the layers carefully each time. Dry the CCl_4 by shaking with plaster of Paris, and decant. Distil the decanted solution and discard the first and last 25 cc. portions.

(h) *Potasium iodide solution.*—0.1%.

(i) *Potassium iodide, standard solution.*—Dissolve 0.654 gram of KI in 100 cc. of water. Dilute 10 cc. of this solution to 100 cc. and 1 cc. to 100 cc. One cc. of this solution contains 5 micrograms of iodine, and 1, 2, or 5 cc. is used in making up the series of standards necessary.

Use for the distillation of the iodine a special all-glass apparatus described in detail, with outline drawing, by Trevorrow and Fashena,[6] and manufactured by the Scientific Glass Apparatus Company, Bloomfield, N. J. The distillate, evolved in a 150 cc. round-bottomed flask, is led through a closely connected train consisting of (1) an inverted 250 cc. Erlenmeyer flask, into the bottom of which is sealed (2) a bulb, into which is sealed a Kjeldahl connecting tube, and (3) a condenser, connected by a ground glass joint to (4) the receiving vessel, cylindrical in shape and containing about 100 cc. All joints are of glass and of standard taper.

PREPARATION OF MATERIAL

Plant material.—Burn sample in the apparatus and in the manner described by von Kolnitz and Remington.[7] Combine the ash and washings with the main volume of the solution, add a few pellets of NaOH, and evaporate the solution to a volume of about 30 cc. Determine iodine in this solution as directed below.

Waters.—Add a few pellets of NaOH to a 500 cc. aliquot of the sample and evaporate to about 30 cc. Proceed as directed below.

Soils.—Weigh the sample of soil directly into a 250 cc. beaker, add about 30 cc. of water, and proceed as directed below.

PROCEDURE

To not more than 30 cc. of solution, add 6 grams of the $KCrO_4$, 30 cc. of the chromic acid-sulfuric acid mixture, and about 10 mg. of the $Ce_2(SO_4)_2$. Heat mixture

[6] *J. Biol. Chem.*, 110, 34 (1935).
[7] *Ind. Eng. Chem., Anal. Ed.*, 5, 38 (1933).

over a slow free flame until temperature is 195° C., shaking occasionally to insure destruction of all organic matter (digestion period usually about 1 hour). Cool, add 50 cc. of water, and filter through an asbestos mat to remove the insoluble material. Prepare the asbestos by carrying it through the same digestion. Cool to 50° C. or lower and transfer solution to the 150 cc. glass distillation flask. Add 10 cc. of the phosphorous acid, shake, and connect to the distillation outfit. Destroy all the yellow color; if necessary add more phosphorous acid. Attach the receiver containing 10 cc. of 0.02 *N* NaOH, start slow suction through the apparatus, and apply heat to the distillation flask (suction should be just sufficient to prevent any back pressure until the solution starts boiling); then increase suction so that the solution is rapidly agitated (50–75 liters of air per hour). Continue distillation until the temperature of the solution is 150°, so regulating the heat of the burner that this will require about 30 minutes. Discontinue the distillation and remove the receiver, taking extreme care at all times to prevent any back pressure in the system.

Transfer solution in the receiving flask to a 150 cc. beaker and acidify (one drop excess) with H_2SO_4 (1+1). Add bromine until the solution turns brown. Boil the solution until the volume is reduced to about 5 cc. Transfer to a small separatory funnel and make up to 10 cc., washing beaker with small amounts of water. Add 4 drops of H_2SO_4 (1+1), 1 cc. of .1% KI solution, and shake. Add 1 cc. of CCl_4. Shake vigorously for 2 minutes. Transfer the CCl_4 to a 15 cc. centrifuge tube and centrifuge at full speed for 1 minute. Compare the color with a standard solution prepared in the same way and containing approximately the same quantity of iodine as the unknown. Make up a series of standards containing 5, 10, and 25 micrograms in order to insure against too great a spread between the standard and the unknown; one of these may be omitted if it is known that the unknown does not contain a similar amount of iodine.

EXPERIMENTAL WORK

Because the volumetric method for the microtitration of very small quantities of iodine with thiosulfate usually gave variable results, the colorimetric method was always used. Two colorimetric procedures were studied: A direct method in which iodine is liberated by the addition of sodium nitrite to the acidified distillate and read directly, and an indirect method in which the iodine in the distillate is oxidized to iodic acid by bromine, the bromine boiled off, potassium iodide added, and iodine liberated by the iodic acid thus formed. With both procedures the liberated iodine was extracted from the solution by carbon tetrachloride and the color was compared with that produced by standard potassium iodide solutions prepared in the same way. If correspondingly accurate results can be obtained, the iodic acid procedure should be better than the nitrous acid procedure, since the quantity of iodine liberated by the iodic acid is six times greater than that liberated by the nitrous acid. This factor is of great importance if very small amounts of iodine are to be estimated. On the other hand, oxidizing substances other than iodine might liberate iodine from the potassium iodide. Experiments were also made to determine whether accurate results could be obtained by a colorimetric adaptation of the iodic acid procedure.

The strength of the potassium iodide solution was first studied. In

the macro method, a 10 per cent solution is usually used in the final liberation of iodine, but it was found that with this strength only a small part of the iodine liberated was extracted by the carbon tetrachloride. Although the aqueous layer in the separatory funnel was colored distinctly yellow by the liberated iodine, the color in the carbon tetrachloride was not much deeper than that produced with the direct procedure by means of sodium nitrite, because the potassium iodide retained a large part of the iodine in the aqueous fraction. A question accordingly arose as to the strength of potassium iodide solution that would reduce all the iodic acid but be sufficiently dilute to keep to a minimum the iodine retained by the aqueous layer. Solutions containing 25 micrograms of iodine were made up and treated in the same way except that the iodic acid was reduced by potassium iodide solutions of different strengths. Iodine was then extracted by carbon tetrachloride, and the depth of color in the different solutions was compared, one of the solutions being used as standard. When the carbon tetrachloride extract from the solution treated with 0.1 per cent potassium iodide solution was placed in one tube of the colorimeter set at 20.0, corresponding readings in the other tube of the colorimeter containing carbon tetrachloride extracts from the solutions treated with 10.0, .075, .050, .025, and .010 per cent potassium iodide solutions were 7.0, 19.8, 19.9, 18.1, and 8.4, respectively. The color from the solution treated with the 10 per cent solution of potassium iodide was only about one-third as deep as that from the solution treated with the 0.1 per cent solution. The solutions treated with .025 per cent and .010 per cent solutions of potassium iodide did not liberate all the iodine, while the solutions treated with 0.1, .075, and .050 per cent solutions of potassium iodide were identical within the limits of accuracy of reading the colorimeter. A 0.1 per cent solution of potassium iodide was, therefore, used in later work.

In preliminary work sulfuric acid (1+1) gave better results than did other acids for the acidification of the solution prior to the addition of bromine. Results obtained by varying the quantity of the sulfuric acid from 4 to 45 drops showed that within the limits of experimental error in reading the colorimeter, these quantities of acid had no effect upon the depth of color.

All of the bromine added to the solution must be removed by boiling since bromine in the final solution adds to the color produced in the carbon tetrachloride. Boiling periods of 4, 7, 12, 17, and 32 minutes showed no difference in the results. Bromine can be completely removed by the prolonged boiling specified in the procedure.

The indirect iodic acid procedure was tested in the recovery of iodine from pure solutions of potassium iodide. A known quantity of standard potassium iodide was added to distilled water by one analyst, and the iodine present was estimated by another (Table 1). The average recovery

in 19 tests, 101.2 per cent, with a standard deviation of 8.3 per cent, is satisfactory for colorimetric work. The quantities found that differed appreciably from those present were in solutions containing less than 10 micrograms of iodine. The average recovery of iodine from 10 solutions containing more than 10 micrograms of iodine was 99.1 per cent, with a

TABLE 1.—*Iodine recovered from known solutions of potassium iodide*

PRESENT	FOUND	RECOVERY	PRESENT	FOUND	RECOVERY	PRESENT	FOUND	RECOVERY
micrograms	*micrograms*	*per cent*	*micrograms*	*micrograms*	*per cent*	*micrograms*	*micrograms*	*per cent*
1.5	1.25	83	10.0	10.95	109	25.0	24.10	96
2.5	3.00	120	11.0	10.70	97	25.0	25.75	103
4.0	4.35	109	15.0	14.90	99	35.0	36.55	104
6.0	7.00	117	17.5	17.85	102	37.5	36.50	97
7.0	6.90	99	19.5	19.40	99	46.5	45.25	97
7.5	7.10	95	20.0	19.40	97	50.0	49.70	99

standard deviation of 2.6 per cent, which is well within the limits of experimental error in colorimetric work. The range in percentage of recovery was from 83.3 per cent in a solution containing 1.5 micrograms of iodine to 120 per cent in a solution containing 2.5 micrograms. The differences between the quantity found and the quantity present were less than 5 per cent in 12 of the 19 cases, and in only 1 case was the difference greater than 10 per cent when the quantity of iodine present exceeded 5 micrograms. This agreement is considered good.

TABLE 2.—*Recovery of iodine added to soils, by iodic acid procedure*

SOIL A (1.5 p.p.m.)			SOIL B (2.3 p.p.m.)			SOIL C (2.4 p.p.m.)			SOIL D (5.0 p.p.m.)		
ADDED	RECOVERED		ADDED	RECOVERED		ADDED	RECOVERED		ADDED	RECOVERED	
micro-grams	*micro-grams*	*per cent*	*micro-grams*	*micro-grams*	*per cent*	*micro-grams*	*micro-grams*	*per cent*	*micro-grams*	*micro-grams*	*per cent*
10	10.0	100	10	11.0	110	10	12.0	120	10	11.5	115
10	8.5	85	20	20.5	102	15	14.5	97	20	21.0	105
10	11.5	115	30	31.0	103	20	19.5	97	20	22.5	112
20	17.5	88	40	38.5	96	20	20.0	100	25	24.0	96
20	17.5	88	50	46.5	93	20	21.0	105	30	33.0	110
20	21.0	105				25	24.5	98	50	51.0	102
20	22.5	112				25	26.5	106	50	43.5	87
35	35.0	100				30	28.5	95			
40	37.5	94				30	31.0	103			

The indirect iodic acid procedure for soils was tested by ascertaining the recovery of iodine added to soils of known iodine content. Varying quantities of standard potassium iodide solution were added to the soils, which were then fused with potassium hydroxide, the iodides extracted with alcohol, and the iodine in the evaporated extracts estimated by the iodic acid procedure. The recovery (Table 2) ranges from 85 per cent to

115 per cent of the quantity added, and the average recovery is 101.3 ± 8.7 per cent. This agreement is also satisfactory.

The indirect iodic acid procedure was then compared with the direct nitrous acid procedure by fusing duplicate portions of soil with potassium hydroxide, extracting the iodides with alcohol, and determining the iodine in one solution by the direct nitrous acid procedure and in the other by the indirect iodic acid procedure (Table 3). The average iodine estimated by the indirect method, when expressed as percentage of that estimated

TABLE 3.—*Iodine content of 18 soils, as estimated by direct method with nitrous acid and by indirect method with iodic acid*

(Results expressed as p.p.m.)

DIRECT	INDIRECT	RATIO	DIRECT	INDIRECT	RATIO	DIRECT	INDIRECT	RATIO
2.3	2.3	100	4.8	3.7	77	9.2	9.8	106
3.4	3.2	94	4.9	4.8	98	12.9	14.8	114
3.4	3.3	97	7.2	7.4	103	13.9	12.4	89
4.2	4.1	98	8.8	8.6	98	13.9	13.8	100
4.4	4.9	111	8.7	8.0	92	14.5	14.7	101
4.6	4.3	94	9.1	8.5	94	15.5	15.5	100

by the direct method, is 98.0 ± 8.0 per cent, which is satisfactory. A similar experiment, in which water samples were used, gave results showing that the agreement between the two methods was as good for water as for soils.

These experiments show that the indirect iodic acid is as accurate as the direct nitrous acid procedure, but the former has the great advantage that a much deeper color is produced in the carbon tetrachloride when the same quantity of iodine is originally present. This makes possible a more accurate comparison with the same quantity of sample, or permits the use of a smaller sample.

In the procedure presented, in which the sample is digested at high temperature with a mixture of chromic and sulfuric acids, all the material from the sample is present in the reacting mixture during the digestion of the sample. The digestion is sufficiently long and severe to insure that, at the completion of the digestion, all the iodine is in solution, and the removal of the insoluble material by filtration at this point in the procedure does not result in any loss of iodine. This was shown by determinations of iodine on duplicate runs, in one of which the insoluble material was left in the solution during distillation, while in the other it was removed by filtration. The agreement between the results of the two runs was entirely satisfactory. The principal advantage gained by the filtration is the freedom from bumping during distillation. Bumping is not serious with most plant materials nor with water low in lime salts, but it is almost always serious with soils. Since there is no advantage to

be gained by not filtering this is recommended because of the increased ease of manipulation.

Digestion with chromic acid is particularly advantageous in the estimation of iodine in soils. It is preferred to procedures in which the iodine is driven off by high temperatures in an electric furnace because it is much more rapid, and also to procedures in which the soil is fused because it requires much less time, and it does not introduce any large amount of salts that must later be removed by extraction. Fusion may also result in liberation of iodine from highly insoluble silicates.

Distillation of the iodine from the main solution offers the important advantage that the iodine is removed from all the interfering substances (such as organic matter, manganese, and chromium) that might be present. Distillation of the iodine has also been proposed by Stimmel and McCullagh[8] and recently by McHargue and Offutt[9] for plant material. The method is also of value for the estimation of iodine in the solutions from soils and water. The modified Trevorrow-Fashena method presented here offers another advantage in that the distillation system is constantly swept out by a current of iodine-free air so that all the iodine evolved is carried over into the absorbing solution. In order to reduce the volume of the distillation system to a minimum the distillation flask should be as small as practicable. In this work a 150 cc. distillation flask was used almost entirely. During the early part of the distillation the rate of boiling must be kept down to prevent carrying over any of the solution, but the inverted Erlenmeyer placed immediately above the distillation flask provides a safety margin for the system in this respect. The temperature of the chromic acid mixture must be below 50° C. before the phosphorous acid is added because the heat of the reaction is sufficient to raise the temperature of the mixture considerably, and unless it is originally below 50° C., some iodine and solution may be lost by sudden and violent boiling. Sufficient phosphorous acid must be added to reduce all the chromic acid; otherwise no iodine will be evolved. Experiments by the writers have shown that a small quantity of phosphorous acid in excess of that required has no detrimental effect other than the unnecessary expenditure of chemicals.

A comparison of the results obtained on four soils by McHargue, who volatilized the iodine from the soil in an electric furnace, and by Fudge, who used the method given here, is shown in Table 4. The results obtained by Fudge are slightly higher than those obtained by McHargue. The greatest difference in the results (0.7 p.p.m.) was in the case of sample No. 36498, in which 14.1 p.p.m. were found by Fudge, and 13.4 p.p.m. by McHargue. However, McHargue[10] states that a difference of only 0.7 p.p.m. is very good agreement for two analysts on the same sample.

[8] *J. Biol. Chem.*, 116, 21 (1936).
[9] *This Journal*, 22, 471 (1939).
[10] *This Journal*, 20, 222 (1937).

Results by different collaborators on the same soils, reported by McHargue in the same paper, show in the case of some soils a much wider spread than this, ranging in one soil from 14.3 to 9.04 p.p.m. It may be concluded that the agreement between the results obtained by the two

TABLE 4.—*Comparison of results by chromic acid digestion and those obtained by McHargue by combustion method*

(Results expressed as p.p.m.)

LABORATORY NUMBER	SOIL	COMBUSTION	ACID DIGESTION
24035	Ruston fine sandy loam	1.1	1.2
36369	Amarillo fine sandy loam	2.2	2.8
23321	Victoria fine sandy loam	4.5	5.1
36498	Houston black clay	13.4	14.1

procedures is fairly satisfactory, which leads to the conclusion that the digestion of the soil with the chromic acid mixture, in addition to some marked advantages in ease of manipulation, is fully as accurate as the method in which the soil is ignited in a furnace.

In another test different quantities of the same samples of water were used, viz., aliquots of 500 cc. and of 2,000 cc. The average for 8 samples containing 4–25 p.p.b. was 15.6 p.p.b. for the 500 cc. aliquots and 16.4 p.p.b. for the 2,000 cc. aliquots. The greatest difference was 4 p.p.b. on a sample containing 20 p.p.b. of iodine in the aliquot of 500 cc. and 24 in the aliquot of 2,000 cc.

SUMMARY

Modified procedures for the estimation of iodine in soils, plant material, and waters are given. The sample (after ignition in the case of plant material) is digested in a chromic acid-sulfuric acid mixture, the iodine is distilled in an all-glass apparatus after reduction with phosphorous acid, and the iodine in the distillate is estimated colorimetrically after oxidation with bromine, reduction with 0.1 per cent potassium iodide solution, and extraction with carbon tetrachloride.

Note on the Kjeldahl Digestion of Sugarcane Juice*

In the digestion of sugarcane juice with sulfuric acid for nitrogen determinations, considerable trouble and loss of time caused by excessive and persistent foaming were almost entirely obviated by the following procedure.

The sugarcane juice sample was prepared for the digestion in the usual manner, 25 ml. of sugarcane juice+35 ml. of concentrated sulfuric acid+

* By L. G. Davidson, *Assistant Chemist*, Division of Soil Fertility Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

10 grams of a mixture of 20 parts of potassium sulfate, 1 part cupric sulfate and 2 parts of ferrous sulfate in a 500 ml. Kjeldahl flask being used, and then heated to boiling or until foaming started. The flask containing the sample was then allowed to stand overnight, and in the morning the digestion was continued in the usual way. In transferring the sugarcane juice to the flask, as little water as possible was used in order to minimize the initial foaming.

Recently this method was used in another laboratory for the digestion of sugar samples, and excellent results were obtained.

This method might be valuable in the digestion of large samples of other highly carbonaceous materials.

BOOK REVIEW

Growing Plants Without Soil. By D. R. MATLIN. Chemical Publishing Co., Inc., New York, N. Y. 1939. 137 pp. Price $2.00.

The writer has read quite thoroughly and with interest the parts of this book that bear primarily on the subject of growing plants without soil, as covered in the first 46 pages. The remainder of the book is devoted to various horticultural subjects including propagation, grafting, construction and operation of greenhouses, flower planting calendar, tables of weights and measurements, etc., and ends up with a list of Agricultural Experiment Stations together with the names of the directors. However, the book would better serve its intended purpose had the author limited himself to the subject of growing plants without soil rather than to have included other information.—W. R. BEATTIE.