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BOOK REVIEWS

PROCEEDINGS OF THE SIXTY-SIXTH ANNUAL MEETING OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1952

The sixty-sixth annual meeting of the Association of Official Agricultural Chemists was held at the Shoreham Hotel, Washington, D. C., September 29, 30, and October 1, 1952.

The meeting was called to order by the President, Henry A. Lepper, on the morning of September 29, at 9:30 A.M.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING OCTOBER 1953

President

H. J. FISHER, Conn. Agricultural Experiment Station, New Haven 4, Conn.

Vice-President

E. L. GRIFFIN, Production and Marketing Administration, Livestock Branch, Washington 25, D. C.

Secretary-Treasurer Emeritus

W. W. SKINNER, Kensington, Md.

Secretary-Treasurer

WILLIAM HORWITZ, U. S. Food and Drug Administration, Washington 25, D. C.

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K. D. JACOB, Beltsville, Md.
M. P. ETHEREDGE, State College, Miss.
H. A. LEPPER, Washington, D. C.

Editorial Board

WILLIAM HORWITZ, *General Chairman*

Editorial Committee of the Journal

PAUL A. CLIFFORD (U. S. Food and Drug Administration, Washington 25, D. C.), *Chairman and Editor* (1955)*

J. B. SMITH (1953)
A. H. ROBERTSON (1955)
E. L. GRIFFIN (1957)
F. H. WILEY (1957)
RUTH D. GROESBECK, *Assistant Editor*

Committees

Committee to Confer with American Public Health Association on Standard Methods of Milk Analysis

A. H. ROBERTSON (Director, State Food Laboratory, Albany, N. Y.), *Chairman*
WILLIAM HORWITZ

* Figures in parenthesis refer to year in which appointment expires.

Committee to Confer with American Society for Testing Materials and Soil Conditioners

F. W. QUACKENBUSH (Purdue University, Lafayette, Ind.), *Chairman*
W. H. MACINTIRE ——— ———

Committee on Spectrophotometric Nomenclature

J. H. JONES (Food and Drug Administration, Washington 25, D. C.), *Chairman*
J. CAROL W. T. MATHIS G. M. WYMAN ———

Committee on Recommendations of Referees

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

SUBJECTS, REFEREES, AND ASSOCIATE REFEREES†

SUBCOMMITTEE A:

J. B. SMITH (1954), (Agricultural Experiment Station, Kingston, R. I.), *Chairman;* C. V. MARSHALL (1956); and C. D. TOLLE (1958).

FEEDING STUFFS:

Referee: M. P. Etheredge, Mississippi State College, State College, Miss.

ASH IN FEEDING STUFFS:
Ralph Willis, N. J. Agric. Experiment Station, New Brunswick, N. J.

CRUDE FAT OR ETHER EXTRACT:
H. H. Hoffman, Department of Agriculture, Tallahassee, Fla.

CRUDE PROTEIN IN FEEDING STUFFS:
E. A. Epps, Jr., La. Department of Agriculture and Immigration, P. O. Box 89, Baton Rouge, La.

DRUGS IN FEEDS:
R. T. Merwin, Agricultural Experiment Station, New Haven, Conn.

FAT IN FISH MEAL:
Maurice E. Stansby, Fish and Wildlife Service, Seattle 2, Wash.

MICROSCOPIC EXAMINATION:
J. A. Shrader, Agricultural Experiment Sta., Lexington 29, Ky.

MILK BY-PRODUCTS IN MIXED FEEDS:
Ara O. Call, Western Condensing Co., 935 East John St., Appleton, Wis.

MINERAL CONSTITUENTS OF MIXED FEEDS:
J. C. Edwards, Department of Agriculture, Tallahassee, Fla.

FERTILIZERS:

Referee: F. W. Quackenbush, Dept. of Agricultural Chemistry, Purdue Univ., Lafayette, Ind.

ACID- AND BASE-FORMING QUALITY:
E. W. Constable, State Department of Agriculture, Raleigh, N. C.

AMMONIACAL SOLUTIONS AND LIQUID FERTILIZERS:
J. F. Fudge, Agricultural Experiment Station, College Station, Texas.

BORON:
Rodney Berry, Va. Department of Agriculture, State Office Bldg., Richmond 19, Va.

† Referees appointed during the year for unassigned subjects will be announced in *The Journal.*

COPPER AND ZINC:
H. J. Webb, A. and M. College of South Carolina, Clemson, S. C.
FREE WATER:
W. L. Hill, Plant Industry Station, Beltsville, Md.
INERT MATERIALS:
K. G. Clark, Division of Fertilizer and Agricultural Lime, Beltsville, Md.
MAGNESIUM AND MANGANESE:
John B. Smith, Agricultural Experiment Station, Kingston, R. I.
NITROGEN:
H. A. Davis, Agricultural Experiment Station, Durham, N. H.
PHOSPHORIC ACID:
K. D. Jacob, Plant Industry Station, Beltsville, Md.
POTASH:
O. W. Ford, Purdue University, Lafayette, Ind.
SAMPLING AND PREPARATION OF SAMPLE:
Stacey B. Randle, N. J. Agr. Expt. Station, New Brunswick, N. J.
SOIL CONDITIONERS:

SULFUR:
Gordon Hart, Department of Agriculture, Tallahassee, Fla.

ECONOMIC POISONS:

Referee: Thomas H. Harris, Production and Marketing Administration, Insecticide Division, Washington 25, D. C.
ALDRIN:
S. J. Few, Miss. State Chem. Laboratory, State College, Miss.
ALLETHRIN:
M. S. Konecky, Bur. Entomology and Plant Quarantine, Beltsville, Md.
BENZENE HEXACHLORIDE:
Irwin Hornstein, Bur. Entomology and Plant Quarantine, Beltsville, Md.
CHLORDANE AND TOXAPHENE:
T. H. Harris, Production and Marketing Adm., Insecticide Div., Beltsville, Md.
DDT AND RELATED COMPOUNDS:
E. E. Fleck, Bur. Entomology and Plant Quarantine, Beltsville, Md.
DIELDRIN:
J. B. McDevitt, Jr., Feed and Fertilizer Lab., Louisiana State College, Baton Rouge 3, La.
DITHIOCARBAMATES:
J. D. Patterson, Department of Agriculture, Salem, Oreg.
HERBICIDES:
W. A. Affens, Production and Marketing Adm., Insecticide Div., Beltsville, Md.
ISOPROPYL PHENYL CARBAMATES AND RELATED COMPOUNDS:

PARATHION:
Paul A. Giang, Bur. Entomology and Plant Quarantine, Beltsville, Md.
PHENOLIC DISINFECTANTS:
Frank A. Spurr, Bureau of Animal Industry, Washington 25, D. C.
PHYSICAL PROPERTIES OF ECONOMIC POISONS:
E. L. Gooden, Bur. Entomology and Plant Quarantine, Beltsville, Md.

PIPERONYL BUTOXIDE:
B. L. Samuel, Div. Chemistry, Dept. of Agriculture, Richmond 19, Va.
PYRETHRINS:
D. Kelsey, Production and Marketing Adm., Insecticide Div., Beltsville, Md.
QUATERNARY AMMONIUM COMPOUNDS:
R. L. Caswell, Production and Marketing Adm., Insecticide Div., Beltsville, Md.
RODENTICIDES:
J. B. LaClair, Department of Agriculture, Sacramento 14, Calif.
ROTENONE:
R. Payfer, Plant Products Div., Dept. of Agriculture, Ottawa, Canada
SYSTEMIC INSECTICIDES:

VOLATILITY OF ESTER FORMS OF HORMONE TYPE HERBICIDES:
E. A. Walker, Insecticide Div., Production and Marketing Adm., Washington 25, D. C.

DISINFECTANTS:

Referee: L. S. Stuart, Production and Marketing Adm., Livestock Branch, Washington 25, D. C.
FUNGICIDES AND SUBCULTURE MEDIA:
L. F. Ortenzio, Agricultural Research Center, Beltsville, Md.
MEDIA FOR DISINFECTANT TESTING:
Michael J. Pelczar, Dept. of Bacteriology, Univ. of Md., College Park, Md

PLANTS:

Referee: E. J. Miller, Agricultural Experiment Station, East Lansing, Mich.
BORON:
E. Truog, Dept. Soils, University of Wisconsin, College of Agriculture, Madison 6, Wis.
CAROTENE:
E. J. Benne, Agricultural Experiment Station, East Lansing, Mich.
COPPER AND COBALT.
Kenneth C. Beeson, U. S. Plant, Soil, and Nutritional Lab, Ithaca, N. Y.
POTASSIUM:

SAMPLING:
E. J. Miller
SODIUM:
Eunice J. Heinen, Mich. Agricultural Experiment Sta., East Lansing, Mich.
STARCH:
Carroll L. Hoffpauir, Southern Regional Research Lab., New Orleans, La.
SUGAR:
Kenneth T. Williams, Western Regional Research Lab., Albany 6, Calif.
ZINC:
E. J. Benne

SOILS AND LIMING MATERIALS:

Referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville 16, Tenn.

BORON:

E. Truog, Dept. Soils, University of Wisconsin, College of Agriculture, Madison 6, Wis.

EXCHANGEABLE CALCIUM AND MAGNESIUM:

W. M. Shaw, Agricultural Experiment Station, Knoxville 16, Tenn.

EXCHANGEABLE HYDROGEN:

W. M. Shaw

EXCHANGEABLE POTASSIUM:

A. Mehlich, N. C. State College of Agr. and Eng., Raleigh, N. C.

FLUORINE:

L. J. Hardin, Agricultural Expt. Sta., Knoxville 16, Tenn.

HYDROGEN-ION CONCENTRATION OF SOILS:

Lannes E. Davis, Div. of Soils, Calif. Agr. Expt. Sta., Davis, Calif.

MOLYBDENUM:

W. O. Robinson, Bur. Plant Industry, Beltsville, Md.

PHOSPHORUS:

L. E. Ensminger, Alabama Experiment Station, Auburn, Ala.

ZINC AND COPPER:

J. G. A. Fiskel, Agricultural Experiment Station, Gainesville, Florida

STANDARD SOLUTIONS:

Referee: H. G. Underwood, Food and Drug Administration, Washington 25, D. C.

CONSTANT BOILING HYDROCHLORIC ACID:

Sidney Williams, Food and Drug Administration, Boston 10, Mass.

SODIUM THIOSULFATE:

W. F. Munday, Food and Drug Administration, Kansas City 6, Mo.

TOBACCO:

Referee: R. N. Jeffrey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md.

NUTRITIONAL ADJUNCTS:

Referee: O. L. Kline, Food and Drug Administration, Washington 25, D. C.

CAROTENE:

F. W. Quackenbush, Dept. of Agricultural Chemistry, Purdue Univ., Lafayette, Ind.

PANTOTHENIC ACID (MICROBIOLOGICAL METHOD):

H. W. Loy, Jr., Food and Drug Administration, Washington 25, D. C.

THIAMINE IN ENRICHED CEREAL PRODUCTS:

L. H. McRoberts, Food and Drug Administration, San Francisco 2, Calif.

VITAMIN A IN OLEOMARGARINE:

Kenneth Morgareidge, Food Research Laboratories, 48-14 Thirty-third St., Long Island City 1, N. Y.

VITAMIN A IN FEEDS:

Maxwell L. Cooley, General Mills Inc., 1081 Twenty-first Ave., S.E., Minneapolis 14, Minn.

VITAMIN B_6 (CHEMICAL):

W. L. Hall, Food and Drug Administration, Washington 25, D. C.

VITAMIN B_{12} (MICROBIOLOGICAL METHOD):

Carl H. Krieger, Wisconsin Alumni Research Foundation, Madison, Wis.

VITAMIN D—POULTRY FEED SUPPLEMENTS:

Leo Friedman, Food and Drug Administration, Washington 25, D. C.

SUBCOMMITTEE B:

Harry J. Fisher (1954), (Connecticut Agricultural Experiment Station, New Haven, Conn.), *Chairman;* G. R. CLARK (1956); and JONAS CAROL (1958).

RADIOACTIVITY:

Referee: A. Schwebel, National Bureau of Standards, Washington 25, D. C.

EMISSION SPECTROGRAPHY:

Referee: W. T. Mathis, Connecticut Agricultural Expt. Station, New Haven 4, Conn.

VEGETABLE DRUGS AND THEIR DERIVATIVES:

Referee: P. S. Jorgensen, Food and Drug Administration, San Francisco 2, Calif.

AMINOPYRINE, EPHEDRINE, AND PHENOBARBITAL:

H. C. Heim, School of Pharmacy, University of Colorado, Boulder, Colo.

QUININE AND STRYCHNINE:

D. J. Miller, Food and Drug Administration, Buffalo 3, N. Y.

SYNTHETIC DRUGS:

Referee: F. C. Sinton, Food and Drug Administration, New York 14, N. Y.

ACETOPHENETIDIN AND CAFFEINE (Chromatographic Separation):

Gordon Smith, Food and Drug Administration, New York 14, N. Y.

AMOBARBITAL SODIUM AND SECOBARBITAL SODIUM (TUINOL ®):

George E. Keppel, Food and Drug Administration, Minneapolis 1, Minn.

AMPHETAMINES:

L. H. Welsh, Food and Drug Administration, Washington 25. D. C.

DIPHENHYDRAMINE AND TRIPELENNAMINE HYDROCHLORIDES (BENADRYL ® AND PYRIBENZAMINE ®):

H. C. Heim, School of Pharmacy, Univ. of Colorado, Boulder, Colo.

METHYLENE BLUE:

H. O. Moraw, Food and Drug Administration, Chicago 7, Ill.

PROPADRINE HYDROCHLORIDE:

A. W. Steers, Food and Drug Administration, Los Angeles 15, Calif.

SPECTROPHOTOMETRIC METHODS:

J. Carol, Food and Drug Administration, Washington 25, D. C.

SULFANILAMIDE DERIVATIVES:

H. W. Conroy, Food and Drug Administration, Kansas City 6, Mo.

STEROID ESTROGENS:

Paul M. Sanders, Food and Drug Administration, Washington 25, D. C.

SYNTHETIC ESTROGENS:

Daniel Banes, Food and Drug Administration, Washington 25, D. C.

MISCELLANEOUS DRUGS:

Referee: Iman Schurman, Food and Drug Administration, Chicago 7, Ill.

ALKALI METALS:

GLYCOLS AND RELATED COMPOUNDS:
Harry Isacoff, Food and Drug Administration, New York 14, N. Y.

MERCURY COMPOUNDS:
R. L. Herd, Food and Drug Administration, St. Louis 1, Mo.

MICROSCOPIC TESTS FOR ALKALOIDS AND SYNTHETICS:
W. V. Eisenberg, Food and Drug Administration, Washington 25, D. C.

ORGANIC IODIDES AND SEPARATION OF HALOGENS:
V. E. Stewart, State Department of Agriculture, Tallahassee, Fla.

PRESERVATIVES AND BACTERIOSTATIC AGENTS IN AMPUL SOLUTIONS:
C. N. Jones, Food and Drug Administration, New York 14, N. Y.

COSMETICS:

Referee: G. R. Clark, Food and Drug Administration, Washington 25, D. C.

COLD PERMANENT WAVES:
John E. Clements, Food and Drug Administration, Washington 25, D. C.

COSMETIC CREAMS:
C. F. Bruening, Food and Drug Administration, Chicago 7, Ill.

DEODORANTS AND ANTI-PERSPIRANTS:
John E. Clements

HAIR DYES AND RINSES:
S. H. Newburger, Food and Drug Administration, Washington 25, D. C.

MASCARA, EYEBROW PENCILS, AND EYE SHADOW:
Paul W. Jewel, Max Factor & Co., 1666 N. Highland Ave., Hollywood 28, Calif.

COAL-TAR COLORS:

Referee: K. A. Freeman, Food and Drug Administration, Washington 25, D. C.

ARSENIC AND ANTIMONY IN COAL-TAR COLORS:
L. S. Harrow, Food and Drug Administration, Washington 25, D. C.

BOILING RANGE OF AMINES DERIVED FROM COAL-TAR COLORS:
L. S. Harrow

ETHER EXTRACT IN COAL-TAR COLORS:
S. S. Forrest, Food and Drug Administration, Washington 25, D. C.

HALOGENS IN HALOGENATED FLUORESCEINS:
J. H. Jones, Food and Drug Administration, Washington 25, D. C.

HEAVY METALS IN COAL-TAR COLORS:
C. Stein, Food and Drug Administration, Washington 25, D. C.

IDENTIFICATION OF COAL-TAR COLORS:
C. Graichen, Food and Drug Administration, Washington 25, D. C.

INORGANIC SALTS IN COAL-TAR COLORS:
K. S. Heine, Jr., Food and Drug Administration, Washington 25, D. C.

INTERMEDIATES DERIVED FROM PHTHALIC ACID:
C. Graichen

INTERMEDIATES IN TRIPHENYLMETHANE DYES:
A. T. Schram, National Aniline Division, P. O. Box 975, Buffalo 5, N. Y.

LAKES AND PIGMENTS:
C. Graichen

NON-VOLATILE UNSULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS:
K. S. Heine, Jr.,
PAPER CHROMATOGRAPHY OF COAL-TAR COLORS:
Doris Tilden, Food and Drug Administration, San Francisco 2, Calif.
SPECTROPHOTOMETRIC TESTING OF COAL-TAR COLORS:
J. H. Jones
SUBSIDIARY DYES IN D&C COLORS:
L. Koch, H. Kohnstamm and Company, 537–555 Columbia St., Brooklyn 31, N. Y.
SUBSIDIARY DYES IN FD&C COLORS:
M. Dolinsky, Food and Drug Administration, Washington 25, D. C.
SULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS:
N. Ettlestein, Food and Drug Administration, Washington 25, D. C.
SULFONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS:
John Walton, H. Kohnstamm & Company, Brooklyn 31, N. Y.
UNSULFONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS:
John O. Millham, Food and Drug Administration, Washington 25, D. C.
VOLATILE AMINE INTERMEDIATES IN COAL-TAR COLORS:
K. S. Heine, Jr.

SUBCOMMITTEE C:

A. H. ROBERTSON (1954), (State Food Laboratory, Albany 1, N. Y.), *Chairman:* S. ALFEND (1956); and F. A. VORHES, JR. (1958).

PROCESSED VEGETABLE PRODUCTS:

Referee: L. M. Beacham, Food and Drug Administration, Washington 25, D. C.
CATALASE IN FROZEN VEGETABLES:
B. M. Gutterman, Food and Drug Administration, Washington 25, D. C.
MOISTURE IN DRIED VEGETABLES:
B. Makower, Western Regional Research Laboratory, Albany 6, Calif.
PEROXIDASE IN FROZEN VEGETABLES:
M. A. Joslyn, College of Agr., Univ. of Calif., Berkeley 4, Calif.
QUALITY FACTORS:
R. D. Lovejoy, Food and Drug Administration, Washington 25, D. C.

COFFEE AND TEA:

Referee: S. T. Colamaria, Food and Drug Administration, Boston 10, Mass.
CHLOROGENIC ACID IN COFFEE:
L. C. Weiss, Food and Drug Administration, Los Angeles 15, Calif.
MOISTURE IN COFFEE AND TEA:
George Schwartzman, Food and Drug Administration, New York 14, N. Y.

DAIRY PRODUCTS:

Referee: Wm. Horwitz, Food and Drug Administration, Washington 25, D. C.
CRYOSCOPY OF MILK:
T. Dubin, Army Medical Center Graduate School, Walter Reed Army Medical Center, Washington 12, D. C.
DETECTION OF FOREIGN FATS IN DAIRY PRODUCTS:
James H. Cannon, Food and Drug Administration, St. Louis 1, Mo.
FAT IN DAIRY PRODUCTS:
Ernest O. Herreid, Univ. of Illinois, Agricultural Expt. Sta., Urbana, Ill.

FAT IN HOMOGENIZED MILK:
Claude E. Hynds, Dept. of Agriculture and Markets, Albany 1, N. Y.
FROZEN DESSERTS:
H. M. Boggs, Food and Drug Administration, Philadelphia 6, Pa.
PHOSPHATASE TEST IN DAIRY PRODUCTS:

PREPARATION OF BUTTER SAMPLES:
A. L. Weber, Food and Drug Administration, New York 14, N. Y.
PRESSURIZED CREAM:
C. G. Cunningham, Food and Drug Administration, Boston 10, Mass.
SAMPLING, AND PREPARATION OF SAMPLE, OF SOFT CHEESES:
Sam Perlmutter, Food and Drug Administration, Minneapolis, Minn.
SAMPLING, FAT, AND MOISTURE IN HARD CHEESES:
Wm. Horwitz

EGGS AND EGG PRODUCTS:

Referee: F. J. McNall, Food and Drug Administration, Cincinnati 2, Ohio
AMMONIA NITROGEN:
E. B. Boyce, State Dept. Public Health, Boston 33, Mass.

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL INDICES):

Referee: W. I. Patterson, Food and Drug Administration, Washington 25, D. C.
ANIMAL FECAL MATTER:
John Thomas, Food and Drug Administration, New Orleans 16, La.
APPLES:
W. O. Winkler, Food and Drug Administration, Washington 25, D. C.
FISH (HISTAMINE):
D. W. Williams, Food and Drug Administration, San Francisco 2, Calif.
FISH PRODUCTS (ACIDS):
Fred Hillig, Food and Drug Administration, Washington 25, D. C.
LACTIC ACID:

PINEAPPLE (DECOMPOSITION, CARBOHYDRATE):
J. F. Weeks, Jr., Food and Drug Administration, New Orleans 16, La.
SHELLFISH:
George McClellan, Food and Drug Administration, Baltimore 2, Md.
SPINACH (SUCCINIC ACID):
H. D. Silverberg, Food and Drug Administration, St. Louis 1, Mo.
STRAWBERRIES:
P. A. Mills, Food and Drug Administration, San Francisco 2, Calif.
STRAWBERRIES (PIGMENTS):
H. P. Bennett, Food and Drug Administration, New Orleans 16, La.
TOMATOES (SUCCINIC ACID):
H. VanDame, Food and Drug Administration, Cincinnati 2, Ohio
URIC ACID IN CEREAL PRODUCTS:
Helen Barry, Food and Drug Administration, New Orleans 16, La.
URIC ACID IN NUTS:
R. A. Baxter, Food and Drug Administration, Los Angeles 15, Calif.

FAT IN HOMOGENIZED MILK:
Claude E. Hynds, Dept. of Agriculture and Markets, Albany 1, N. Y.
FROZEN DESSERTS:
H. M. Boggs, Food and Drug Administration, Philadelphia 6, Pa.
PHOSPHATASE TEST IN DAIRY PRODUCTS:

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Sam Perlmutter, Food and Drug Administration, Minneapolis, Minn.
SAMPLING, FAT, AND MOISTURE IN HARD CHEESES:
Wm. Horwitz

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Referee: F. J. McNall, Food and Drug Administration, Cincinnati 2, Ohio
AMMONIA NITROGEN:
E. B. Boyce, State Dept. Public Health, Boston 33, Mass.

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL INDICES):

Referee: W. I. Patterson, Food and Drug Administration, Washington 25, D. C.
ANIMAL FECAL MATTER:
John Thomas, Food and Drug Administration, New Orleans 16, La.
APPLES:
W. O. Winkler, Food and Drug Administration, Washington 25, D. C.
FISH (HISTAMINE):
D. W. Williams, Food and Drug Administration, San Francisco 2, Calif.
FISH PRODUCTS (ACIDS):
Fred Hillig, Food and Drug Administration, Washington 25, D. C.
LACTIC ACID:

PINEAPPLE (DECOMPOSITION, CARBOHYDRATE):
J. F. Weeks, Jr., Food and Drug Administration, New Orleans 16, La.
SHELLFISH:
George McClellan, Food and Drug Administration, Baltimore 2, Md.
SPINACH (SUCCINIC ACID):
H. D. Silverberg, Food and Drug Administration, St. Louis 1, Mo.
STRAWBERRIES:
P. A. Mills, Food and Drug Administration, San Francisco 2, Calif.
STRAWBERRIES (PIGMENTS):
H. P. Bennett, Food and Drug Administration, New Orleans 16, La.
TOMATOES (SUCCINIC ACID):
H. VanDame, Food and Drug Administration, Cincinnati 2, Ohio
URIC ACID IN CEREAL PRODUCTS:
Helen Barry, Food and Drug Administration, New Orleans 16, La.
URIC ACID IN NUTS:
R. A. Baxter, Food and Drug Administration, Los Angeles 15, Calif.

GELATINE, DESSERT PREPARATIONS, AND MIXES:

Referee: Sumner C. Rowe, Food and Drug Administration, Washington 25, D. C.

GELATINE AND GELATINE DESSERTS (COMPOSITION):
Joseph H. Cohen, General Foods Corporation, Woburn, Mass.

FISH AND OTHER MARINE PRODUCTS:

Referee: Menno D. Voth, Food and Drug Administration, Seattle 4, Wash.

SALT AND SOLIDS IN OYSTERS:
John P. Traynor, Food and Drug Administration, Baltimore 2, Md.

TOTAL SOLIDS AND ETHER EXTRACT IN FISH AND MARINE PRODUCTS:
H. M. Risley, Food and Drug Administration, Seattle 4, Wash.

GUMS IN FOODS:

Referee: M. J. Gnagy, Food and Drug Administration, Los Angeles 15, Calif.

CACAO PRODUCTS:
Flora Y. Mendelsohn, Food and Drug Administration, Los Angeles 15, Calif.

CATSUP AND RELATED TOMATO PRODUCTS:
T. E. Strange, Food and Drug Administration, Portland, Oreg.

CHEESE (ALGINATES):
M. J. Gnagy

DRESSINGS FOR FOODS:
M. J. Gnagy

FROZEN DESSERTS:
Shirley M. Walden, Food and Drug Administration, Baltimore 2, Md.

MEAT AND MEAT PRODUCTS:

Referee: Roger M. Mehurin, Meat Inspection Div., Bur. of Animal Industry, Washington 25, D. C.

CHEMICAL TESTS FOR IDENTIFICATION OF MEATS:
C. E. Hynds, State Food Laboratory, Albany 1, N. Y.

CREATIN IN MEAT PRODUCTS:
J. M. McCoy, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.

MOISTURE AND FAT IN MEAT PRODUCTS:
Ernest Windham, Vet. Division, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C.

SEROLOGICAL TESTS FOR IDENTIFICATION OF MEATS:
Paul J. Brandly, Bureau of Animal Industry, Beltsville, Md.

STARCH IN MEAT PRODUCTS:
R. A. Chapman, Dept. National Health and Welfare, Ottawa, Can.

MICROCHEMICAL METHODS:

Referee: C. L. Ogg, Eastern Regional Research Lab., Philadelphia 18, Pa.

ELEMENTAL ANALYSIS:

C. L. Ogg

GROUP ANALYSIS:

A. Steyermark, Hoffman-LaRoche, Inc., Nutley, N. J.

NUTS AND NUT PRODUCTS:

Referee: A. M. Henry, Food and Drug Administration, Atlanta 3, Ga.

FREE FATTY ACIDS:

SHREDDED COCONUT (GLYCOLS AND GLYCEROL):

A. J. Shingler, Food and Drug Administration, New York 14, N. Y.

OILS, FATS, AND WAXES:

Referee: Gardner Kirsten, Food and Drug Administration, New York 14, N. Y.

ANTIOXIDANTS:

S. Kahan, Food and Drug Administration, New York 14, N. Y.

PEANUT OIL:

A. B. Karasz, Dept. Agriculture and Markets, Albany 1, N. Y.

SPECTROPHOTOMETRIC METHODS:

David Firestone, Food and Drug Administration, New York 14, N. Y.

SPICES AND OTHER CONDIMENTS:

Referee: E. C. Deal, Food and Drug Administration, New Orleans 16, La.

PREPARATION OF SAMPLE OF FRENCH DRESSING:

A. F. Ratay, Food and Drug Administration, Cincinnati 2, Ohio

SEEDS AND STEMS IN GROUND CHILI:

A. N. Prater, Gentry, Inc., Los Angeles 54, Calif.

SORBITOL:

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VINEGAR:

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ENZYMES:

Referee: J. W. Cook, Food and Drug Administration, Washington 25, D. C.

ENZYMIC METHODS FOR INSECTICIDES.

J. W. Cook

SUBCOMMITTEE D:

FLOYD ROBERTS (1954), (State Laboratories Dept., Lock Box 900, Bismarck, N. D.), *Chairman;* KENNETH L. MILSTEAD (1956); and ROBERT A. OSBORN (1958).

ALCOHOLIC BEVERAGES:

Referee: R. L. Ryan, Laboratory Division, Bureau of Internal Revenue, Washington 25, D. C.

CORDIALS AND LIQUEURS:
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DISTILLED SPIRITS:
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HIGHER ALCOHOLS IN DISTILLED SPIRITS BY CHROMATOGRAPHY:
Alex P. Mathers, Bur. Internal Revenue, Washington 25, D. C.

INORGANIC ELEMENTS IN BEER:
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MALT BEVERAGES, SIRUPS, EXTRACTS, AND BREWING MATERIALS:
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METHANOL:
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CACAO PRODUCTS:

Referee: W. O. Winkler, Food and Drug Administration, Washington 25, D. C.

CACAO INGREDIENTS:
W. O. Winkler

LACTOSE:
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LECITHIN:
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MALT SOLIDS:
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BROMATES IN FLOUR:
W. L. Rainey, Commander-Larabee Milling Co., Minneapolis, Minn.

EGG CONTENT OF CEREAL FOODS:
V. E. Munsey
MILK SOLIDS AND BUTTERFAT IN BREAD:
V. E. Munsey
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W. V. Eisenberg, Food and Drug Administration, Washington 25, D. C.
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PRESIDENT'S ADDRESS*

METHODOLOGY

By HENRY A. LEPPER (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

According to the program before us, we are here attending the Sixty-Sixth Annual Meeting of the Association of Official Agricultural Chemists. In the parlance familiar to so many of us this is a mis-branding. Our Association is older than the implied 66 years. This failure of perfect accord is not serious however, as the inception of the Association in 1884 makes it but two years older. The close agreement of the numeral on the program and the real age shows the importance with which our predecessors and our members, during the years, have considered the perfection of methods. Once each year was not regarded as too frequent to meet to keep the methods abreast of latest scientific developments, many of which have been the result of researches of individual members of the Association. This seriousness of purpose to keep the methods up to date is evidenced by the fact that it required events of no less magnitude than World Wars to cause three annual meetings to be called off in our history. Through all the years our Association has held steadfast to its heritage of dedication, as quoted from its first constitution, "To secure uniformity and accuracy in methods and results of analysis." Thus it has become a society of scientific achievement. It even fulfills what appears lately to have become a test of a true scientific society, and that is the continued publication and support of a scientific Journal otherwise insolvent.

It is no small honor to be accorded the presidency of such a scientific body. It would be untruthful not to admit the pleasure experienced on being so chosen. With me the appreciation of the honor bestowed was accompanied by contemplations not so pleasant, namely the recognition of an obligation to be fulfilled and the realization of time's imprint.

I realized middle age was upon me, for in our Association rarely, if ever, is it the privilege of youth to become president. Middle age has been defined by a glib-tongued radio commentator as "that time of life when you think you will feel better tomorrow than you do today, but you don't." The passage of time, however, is not without its compensations, for it provides experiences to draw on to meet that obligation which brings me before you today; this presidental address.

The first knowledge that there was an A.O.A.C. came to me when I began the study of quantitative analysis. My chemistry professor was

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Dr. Charles E. Munroe who had taught chemistry while at Harvard University to the man who needs no introduction here, Dr. Harvey W. Wiley. They became close friends. Dr. Munroe was outspoken in his admiration for Dr. Wiley and the work he was doing. It was not strange therefore that his students were required to obtain a copy of the Bureau of Chemistry Bulletin No. 107 for use as a supplemental text. The impressive title "Provisional and Official Methods of Analysis of the Association of Official Agricultural Chemists" promised a wealth of analytical methods, which to us at that time was more than fulfilled by the text. I well recall our disappointment over the completeness of the book. Apparently procedures had been perfected for all important foods and determinations. To us with the narrow vision of those just at the doorstep of the profession the question arose as to what opportunity could there be for further research and method development. It was several years later that I learned that such fears were entirely too pessimistic.

My first real experience with the methods of the Association came to me shortly after I was appointed as an assistant chemist in the Bureau of Chemistry. It was my privilege to be assigned to work with Dr. Paul B. Dunbar who by that time had contributed numerous methods useful in law enforcement. He was the Association's referee on fruit products. The call to an administrative career, then already begun, left him little time for active work at the chemist's bench. He asked me if I wished to study methods for fruit acids with him. He stated that the Schmidt-Hiepe method for tartaric, citric, and malic acids on page 80 of Bulletin 107 was worthless, and more accurate and specific methods were needed. The shock of this surprise was reflected in my question—"What, a method in that book, Doctor?" I soon learned there was nothing unusual about the replacement of outmoded methods by those based on the latest developments and improvements in analytical procedures. From that incident of my initiation into the workings of the A.O.A.C. I have devoted much of my active career to the problem of methods and its various ramifications. I propose therefore to draw on this experience to offer observations which may be helpful to the continued progress and prosperity of our Association and the service rendered by it to the continuing successful enforcement of regulatory laws.

It is not my premise that our activities are devoted to the development of methods solely of regulatory application. The history of the Association shows that it has also served in the perfection of methods designed for research in agricultural chemistry. These two objectives provide no basis for sharp demarcation between the many methods. Each field of activity profits by and draws from the other. It is my purpose however to limit my remarks to the regulatory aspects of our methods.

Most of the laws with which the majority of us are concerned require objective evidence for successful enforcement. It is the analyst who as a

result of his examination testifies as to composition, presence of constituents, or ingredients, or to other factors significant in the application of the provisions of the law. Where interpretation is necessary to prove the violation, either he or another scientist depends upon the experience and knowledge obtained by a study of the composition of authentic materials. Without such evidence and testimony failure is most likely on the first obligation of the prosecution, that is, the establishment of a prima-facie case.

The principles of practice in American courts do not favor the use of methods which carry a suspicion of secrecy or can be challenged as untried. This does not mean that there are not exceptional circumstances where methods employed must remain secret until the trial. The courts recognize when there is merit in such situations. However, in a number of cases judges have commented adversely when publicity had not been given methods in advance of their use in support of a case. Generally no comment on this feature is included in the formal opinion of the court. There is one exception which will be discussed later. It is in cases where violation may be avoided by analysis before the goods are brought within the jurisdiction of the law or where analysis is made to fulfill labeling requirements that the methods of regulatory control should be available to those who are in such need of them. It is the right of the individual to be furnished methods by which his product is to be judged. But it is far more important that the method give correct results in the hands of competent analysts. Unless the methods have been tried and proven in this latter respect their use by the prosecution must inevitably face a charge of unreasonableness.

I recognize with you that there is nothing new in these thoughts. They are as old as our Association and involve the fundamentals which led to its inception. They bear repetition to educate our younger members and to remind others of the obligations to be faced in the choice and use of methods in enforcement. It might appear that such reminder is not needed, but, frankly, I recall when expediency too often has led to the attitude that "It is only A.O.A.C. work," as an excuse for setting aside investigations on the development of methods. Methods adequate to meet the demands of jurisprudence are paramount to the work of the enforcement team of scientists, inspectors, and administrative officials. Methods have been called the tools of enforcement. Our Association is the workshop wherein they are patterned, molded, machined, and sharpened into effective instruments. Its functions were summarized by Commissioner C. W. Crawford in his address before us last year* when he said "This Association has established itself throughout its history of 67 years as a forum wherein all concerned may in an atmosphere free from influences other than those of purely scientific approach achieve the common goal—the selection of workable and serviceable methods to maintain the

* *This Journal*, 35, 37 (1952).

safety and wholesomeness and integrity of the nation's supplies of such vital commodities as its foods and drugs." To this I wish to add my humble belief that were there not an A.O.A.C. there would have to be devised some form of organization, or some means, as yet obscure, for accomplishing its functions.

With proceedings in courts involving cases brought under regulatory laws, the important part played by the evidence furnished by methods has been in many instances the subject of discussion by judges in decisions and charges to the juries. The record of Federal cases shows that time and again judges have dealt in great detail with the results and interpretations of analytical evidence. Victories for enforcement far exceed defeats. This no doubt is also true of state cases. My failure to also cite state records is not because of any lack of appreciation of the importance of our methods to State law enforcement, but the decisions on cases under the Federal Food Laws happen to be readily available in one compilation, and there is a limit to the time to be spent, even for the preparation of a Presidential address.

It is notable that with few exceptions in none of the decisions is it mentioned that the methods were those of the A.O.A.C., nor is the Association referred to, although those of us familiar with the details know that frequently such was the case. In fact, I have been present in court on numerous occasions where the prestige of the Association was a factor in the weight of the evidence supplied by its methods. The opposition is quick to capitalize on the lack of Association recognition of methods employed in any case. One case which had its humorous side was the first contest involving the use of the Fitelson test for tea seed oil as an adulterant of olive oil. The contest on a seizure came up before opportunity was found to study the method collaboratively. In an attempt to support the accuracy of the test I was called upon to give evidence of the successful outcome of my independent study of the method. During the cross-examination the opposing lawyer tried to make much over the fact that the A.O.A.C. had not adopted the method. I explained there had been no meeting of the Association since the method had been perfected and hence no opportunity to adopt it. I went on to assure the counsel that at the next meeting the Association would adopt it—and at this point the judge in no uncertain terms warned me not to attempt to be a prophet in his court. Little did he realize the accuracy with which such a prediction could be made, and in spite of his admonition the Association did adopt the method at its next meeting. We won the case.

There were two cases, at least, where the judges referred specifically to the Association. By coincidence, they were both on eggs and both were lost. One was a prosecution on a shipment of frozen eggs. The evidence to support the charge of decomposition was based solely on the smell test. In his decision the judge referred to certain facts disclosed in the evi-

dence and of which he said he had knowledge which he could not overlook in deciding whether the Goverment had sustained the burden of establishing its case beyond a reasonable doubt. One of these facts stated was: "I do know that for years chemists have been seeking more efficient and rigid methods for the determination of the presence of decomposition in eggs. One need only study the reports of the Association of Official Agricultural Chemists to become aware of this effort. See *Journal of the Association of Official Agricultural Chemists*, Vol. XX, p. 159 (1937); Vol. XXI, p. 179 (1938); Vol. XXII, p. 298 (1939); Vol. XXIV, p. 119 (1941); Vol. XXIV, p. 319 (1941). In most of these studies, representatives of the Food and Drug Administration participated either as referees or associate referees. It is difficult for me to believe that if the organoleptic test is as efficient as plaintiff's witnesses say that such complete and consistent efforts were being made by the chemists to acquire rapidity in their processes."

The other case was a contested seizure on dried eggs charged as being decomposed. In that case the evidence rested on bacteriological and chemical results. In his decision the judge quoted from the above quotation in the frozen egg case down to the word "effort" and then went on to say "The tests upon which the government here relied were developed in secret. The experimenters did not disclose the methods used in their tests or their conclusions either to the Association of Official Agricultural Chemists or to any other scientific society. Furthermore, they did not announce these tests or their conclusions to the industry. They were 'sprung' on the claimant herein and apparently in one other similar action tried about the same time." (I should like to interject at this point that the other similar action resulted in a favorable decision for the Government.) "In view of the long efforts to try to attain some reliable standards. it would have been only fair to all concerned for such tests and conclusions to have been disclosed to the industry, so that an opportunity would have been afforded to verify them, or to determine whether the arbitrary limits stated by the department were proper conclusions to be drawn from the tests. This is especially true as all decomposition and fermentation in foods is not undesirable. Roquefort and other cheeses, and sauerkraut are examples.

"The high bacteria count for the authentic pack indicated in the Government tests resulted only when good eggs were contaminated and held at an 85° temperature for 18 hours or more. The evidence discloses that the claimant herein followed no such practice. It used fresh, current receipt eggs which had been inspected by candling. They were kept at an ideal temperature up to the time when they were dried. This positive testimony offsets the expert opinion of the government witness which was based upon an experiment which was never submitted to the Association of Official Agricultural Chemists or to other learned scientific societies."

Although the work of our Association and its methods did not contribute to victory in these cases, their mention has a significance to us. In the frozen egg case, there is the implication that had scientific evidence been presented based on investigations reported by members of this Association in support of testimony of the Government witnesses on the significance of the smell of the eggs, it would have been adequate to overcome the contradictory testimony of experienced egg dealers that the eggs did not have the odor of decomposition. In the dried egg case, the court, by citation from the text of the court in the other case, recognized the potential weight of scientific evidence. His quarrel was with what he called the secrecy under which the methods were held. Thus did the court in the formal opinion lightly dismiss the preponderance of evidence furnished by the analytical results, so that the testimony of the claimant as to his practices carried more weight. The important factors in the case were more simply summarized at the close of the trial when it was said that although the Government's scientific evidence was unrefuted the man who made the dried eggs testified that nothing happened to them and the court had to believe him. The reference to the A.O.A.C. in this case might imply that had the methods been adopted by our Association the outcome might have been different. On this we can only speculate, but we can take the reference as a recognition of the Association as the authoritative scientific body for the establishment of methods of regulatory application. The lesson to be learned from these two cases is that the activities of our Association should be so conducted as to prevent opportunism from citing it or its work to the detriment of law enforcement.

The responsibility for successfully carrying on the work of the Association rests largely on the referees, including associate referees, and the collaborators. Nothing is to be gained here by repetition of the suggestions, instructions, and recommendations for increased efficiency in referee activity which have been offered in addresses and reports at annual meetings from time to time. However, all who are sincerely interested in carrying this work forward, from the most experienced to the most recently appointed referee, can profit by reading in the pages of our *Journal* from the writings of R. E. Doolittle (*This Journal* **8**, 229 (1925)), E. M. Bailey (*This Journal* **17**, 42 (1934)), and Wm. F. Reindollar (*This Journal* **28**, 38 (1945)), to mention those I now recall who have made contributions of special helpfulness.

It is not intended to chide or criticize, but even as outstanding as the progress has been there are still opportunities for improvement. Year after year one serious handicap to our work is the lateness with which too many referees submit reports. Procrastination is often the reason. Another reason is that unless the need for the method is in the nature of an emergency, other duties are often regarded as more pressing and allowed to take precedence over work on methods. Both causes can be

avoided by systematic planning of the work and allocation of time to be devoted to it. The studies projected each year are never academic. They are of practical application to the needs of today or the demands of tomorrow. Some pressing problems are only those of tomorrow because of lack of methods to handle them today. Every regulatory official has the responsibility to help provide adequate methods to meet all the needs. Wherever methods are wanting, enforcement will stagnate.

While we may take pride in the size of our Book of Methods, may I say that that in itself presents a sizeable problem. Through various economies with space we have been able to keep the methods within the bounds of one volume. Business principles dictate that this be continued if possible. Some referees, but not all, take advantage of the cross reference system used in the book and thus save space through adaptation of available methods to new problems. It has been suggested that a post in the nature of a Director of Referees or Coordinator of Methods be established whose duties would be to bring the methods into closer coordination during the years of development and adoptions between revisions of the Book of Methods. As an example, there are numerous opportunities to unify directions differing not in fundamentals but only in wording. An important function this official might perform would be to serve as a clearing house in advance of changes in methods in one chapter in such cases where they serve other chapters by cross-reference, to provide ample opportunity to the other referees concerned to determine the applicability of the changes or provide other more suitable directions. It is believed that referees would welcome assistance and guidance in some of these responsibilities. According to our two year rule for adoptions the position should be created this year if it is to be helpful for the eighth edition of the Book of Methods.

This Association was the first, or certainly among the first, to use collaborative studies on analytical methods to demonstrate concordance and reproducibility of results. Concordant results on like samples show that the directions that have been studied are adequate, clear, and instructive and that their techniques are sound, workable, and specific. The referees do not always realize that the directions demonstrated by collaborative tests to have these attributes are the wording which merits adoption. Attempts at revision after the study should be avoided, because what is being adopted is the written word collaboratively proven to be adequate to carry all essential instructions. There is no assurance that the same acceptable collaborative results will be obtained by revised directions. The referee should perfect the wording in a precise, clear, expository form before asking collaborative assistance. If revisions are later found to be desirable the method should be restudied.

The question as to who should be collaborators is of major importance. I have said that the methods must give reproducible results in the hands

of competent analysts. Collaborators must not be chosen or assigned without regard to qualifications or background. Many good methods have failed of adoption or their acceptance too long postponed because of poor results obtained by one or more inexperienced analysts during a collaborative study. From their very nature our methods may not be workable by the neophyte or novice in analytical chemistry. On the other hand, they should not require the skills of those of outstanding analytical expertness. There appears to be a happy middleground. Each collaborator should be one whose results would be accepted as correct when obtained with current methods on the same level of difficulty.

It has been my aim to present several observations on methods and their relation to a successful regulatory program. What I think they show can be summarized by a few remarks. Methods, in a vast majority of trials, supply the essential proof. There are well defined and established requirements that methods must meet to stand the test of scrutiny applied in the courts. Methods development, perfection, and modernization should not be left to the will, moods, and scientific curiosity of the laboratory worker. Every organization having laws to enforce which require the use of analytical methods should provide in its regulatory program for a project on methods development. The time allotted to such work should be in proper perspective to the over-all needs of the service. Having been provided, only emergencies of serious threat to the public should curtail that time. Certainly methods work is as important as routine case development. Enforcement activities have been likened to a hare and hound relationship. Violators take the jump and enforcement officials chase and follow. When one is apprehended there is always another in the offing. New and better methods will always be needed. To the extent that methods fail or are wanting, to that extent does the enforcement official begin to go out of business.

IMPORTANT APPLICATIONS OF OUR GROWING KNOWLEDGE OF NUTRITION*

By L. A. MAYNARD (Director, School of Nutrition, Cornell University, Ithaca, N. Y.)

My first full-time job after graduating from college was in feed and fertilizer control. It was about 35 years ago that I first attended a meeting of this Association. As I have worked in the field of nutrition in the years since, I have often thought how the agricultural chemist has participated in the discoveries which have occurred over the years. Your Association has made large contributions to the over-all field of food and nutrition science through the methods which you have developed.

From my own experience and contacts, I know that these published methods have been the "bible" of a host of investigators; the tools which have made possible many of their discoveries. From my contacts with foreign scientists, both in their own laboratories and in their visits to my own, I know that these methods are used much more widely than you may appreciate. I am particularly led to say these things because of my realization of the important role your methods have played in the developments I shall discuss.

Back in 1915 feeding standards for both man and animals took account only of protein, and food energy needs. As to minerals, salt was the only one regularly taken into account in feeding practice. Some quantitative data were available on calcium, phosphorus, and iron requirements for maintenance in man and for calcium and phosphorus needs of cattle. It was assumed that these minerals would normally be adequately furnished in the customary rations. The occurrence of several other mineral elements in both food and body tissues was known but there was not definite proof of their nutritional significance. While the therapeutic value of iodine for goiter was recognized in the middle of the last century, the modern use of iodized salt began in this country around 1920. Though the development of the vitamin concept had resulted in the discovery of vitamin A in 1913, it was many years before quantitative data as to body requirements or the occurrence in foods were available for any vitamin.

At the turn of the century, it was clearly recognized that gelatin could be made more effective in protein nutrition by supplementing it with certain amino acids, and in 1914 Osborne and Mendel showed that the nutritive failure resulting from feeding certain proteins to rats could be overcome by adding missing amino acids. However, these developments were not reflected in feeding practice until much later. In 1915 any consideration of protein was limited to the distinction between "crude" and "true," on the basis that non-protein nitrogen was of no value.

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., September 29, 30, and October 1, 1952.

Thus, in 1915, the recognized nutritional needs of animals and man consisted of six nutrients, plus a source of food energy. The importance of digestibility was understood, as was indicated by the use of digestion coefficients and by the use of the determination of crude fiber in evaluating both human and animal diets. But despite the limited specific knowledge of 35 years ago, the probable discovery of additional dietary essentials was clearly visualized. Impetus was thus given to the nutrition research which has continued at an accelerating rate. We know today that there are some 50 essential dietary constituents.

Both the scientific and practical limitations of such a generalization need to be borne in mind. No such number of dietary essentials are needed for any one species or function. Vitamin C is required by man but not by farm animals. Glycine is needed by chicks but not by mammals. The essentiality of several of the more recently announced vitamins has been shown only in the case of certain lower forms, and even here some of them are still in debate. Moreover, proof that an element or compound is a dietary essential for man or a specific farm animal does not necessarily mean that the factor needs consideration in evaluating their foods and rations. It may always be supplied adequately whatever the makeup of the ration consumed, as is the case for potassium, for example. These facts mean that, for application in feeding practice and feed evaluation, the knowledge represented by the generalization regarding some 50 essentials must be factored out in terms of a given species and function. Our success in feeding a child or chick today depends on the extent of our quantitative knowledge of the nutrients needed by it and also upon quantitative data as to the supply of these nutrients in the foods available for use. From this standpoint, the development of reliable analytical procedures for a given nutrient, and their application to the foods in question, is just as important as the establishment of quantitative requirements. In fact, such procedures are frequently essential for application to test rations or ingredients in the course of arriving at the requirements, as well as for determining the content of the nutrient in the food supply.

The chemist and the feed control official are most immediately interested in those recently discovered nutrients which, on the basis of present information, appear to have practical importance as constituents of feeds and rations. They are also interested in an evaluation of the new research findings as they appear. For these purposes, a development of the last decade is of special significance. Here I refer to the activities of committees of the National Research Council in evaluating the background data and in setting forth recommended allowances of nutrients for both man and farm animals.

The Recommended Dietary Allowances for man were first issued by the Food and Nutrition Board of the Council in 1941. Quantitative data were set forth for both children and adults, for six vitamins and two minerals,

as well as for the calories and protein specified by previous standards. The status of information with respect to various other nutrients was discussed. This original report has been revised and expanded three times as additional data have become available. These reports reflect a continuing program of study by a group of experts and provide assurance that new findings will be translated into practical recommendations as rapidly as the facts justify. A similar job is being done for farm animals. The Committee on Animal Nutrition issued, in 1944–45, Recommended Nutrient Allowances, for poultry, swine, dairy cattle, beef cattle and sheep. All reports were revised in 1949–50 and one for horses was added to the list. Subcommittees are now considering what additional revisions may be called for by current data.

I know that most of you are familiar with these reports. I should like to stress a few points regarding them which seem to me to have special significance. In the first place, they serve to indicate the gaps in our knowledge and stimulate research accordingly. Of special importance to those concerned with dietary and feeding practice, and I believe to your organizations also, these reports have found general acceptance. The obvious advantage of having a single set of figures in general use and the recognition that they are based on thorough deliberations by a representative group of expert scientists have outweighed individual doubts regarding the interpretation of the incomplete data on which some of the values are necessarily based. It is significant that the recommendations are taken account of by both the food and feed industries in marketing the products with which you have to deal.

The practical importance of a quantitative figure as to the need for a givene nutrient cannot be assessed until the quantitative distribution of the nutrient in the foods and rations customarily consumed is known. It is in this connection that much of the information available in the reports on allowances for man and animals needs supplementation for practical use. This is an area in which the A.O.A.C. is making a large contribution and in which much more needs to be done, as I am sure you realize. For the most rapid advancement of feeding practice, data on food composition must keep pace with data on nutrient requirements. This is no routine task. The careful research which your group has found essential for the development of reliable methods applicable to a wide range of products bears witness to that fact. Development and testing of methods for newly discovered nutrients represents an important part of nutritional advances. From the knowledge which is jointly obtained regarding body requirements and food supply, will develop the list of nutrients with which the control official must regularly deal.

I should like to turn to a consideration of the possible impacts on control activities of the recent developments with respect to specific groups of nutrients. Here I tend to enter the realm of speculation and my remarks should be considered accordingly.

In the first place, what is likely to be the application in feeding practice of the current results and active research dealing with amino acid nutrition? The recognition in 1915 that the amino acid makeup of proteins influenced their nutritive value has been mentioned. In succeeding years both laboratory and farm animal studies resulted in establishing numerical ratings for the "biological value" of various protein sources. The modern advances date from 1930 when W. C. Rose introduced the technique of feeding diets in which all of the nitrogen was supplied by amino acids. These studies enabled him to classify ten amino acids as essential for rat growth and ten as non-essential. Extending the studies to man, Rose found eight essential for maintenance and the others dispensable. The studies with rats stimulated similar studies with chicks and pigs. Studies of quantitative requirements followed, with the result that reliable figures are now available for rat and chick growth and human maintenance, and partial data have been reported for pig growth.

It became recognized, however, that an adequate supply of the essential amino acids is not the whole story. They comprise only about 50 per cent of the acids found in body protein. As attention was given to the 10 non-essentials in the case of the rats, it was found that, apart from the cases of cystine and tyrosine, the dietary need was primarily for non-specific sources of nitrogen such as glutamic acid, ammonium citrate, or even urea. These compounds furnished amino groups for the body synthesis of the non-essential acids by combining with compounds arising from carbohydrate or fat metabolism. Experiments with chicks suggest that the same may be true for this species. On the basis of this incomplete evidence, it appears that the protein needs of non-ruminants can be met by specific amounts of essential amino acids plus nitrogen in any form which can provide amino groups for the synthesis of the non-essentials. Any such general conclusion may be premature but, clearly, the emphasis of 1915 on the primary importance of true protein has been reversed.

Do these developments indicate that eventually the protein or nitrogen component of rations will be chosen primarily on the basis of feeding standards setting forth requirements for amino acids, and from data on their content in the feeds available? For various physiological, as well as practical reasons, I don't think this will be the outcome. It is clear, however, that accurate data on the content of the various essential amino acids in feeds and other sources of nitrogen are going to be of increasing importance as a guide to selecting more efficient and more economical rations in the future. The establishment of more precise methods for the determinations called for and the accumulation of representative data on the food supply is a large assignment which recent developments have given to the food scientist. It seems likely that such data will show that only a few of the essentials for a given feeding operation need practical consideration, because most of them will be supplied adequately in any ration selected from the available feeds. Complete data will provide a

better basis than we now have for combining proteins to insure an adequate supply of those acids which might otherwise be deficient, with a minimum use of the more costly protein sources. They may also serve to discover new sources of protein which would supplement our cereals more effectively and more cheaply than is now the case.

Large advances are being made by the chemical industry in lowering the cost of production of certain amino acids now used only on an experimental basis. It may prove in the future to be both good nutrition and good economy to supplement some of our relatively cheap, but low quality sources of protein for animal feeding with one or more essential amino acids in which they are markedly deficient. It is to be hoped that any developments along these lines will be based on decisions of nutrition scientists and regulatory officials so that they are nutritionally and economically sound, and will not result primarily from promotion by those who have the products to sell. The indiscriminate fortification of either human or animal diets with amino acids is to be avoided because of the demonstrated harmful effects of an excess or imbalance.

As you are aware, the developments in the protein nutrition of ruminants have followed quite a different course from those I have just described. The comparisons of protein sources with rats, pigs and chicks in the period following 1915 in turn stimulated similar studies with cattle and sheep, with less definite results. The belief grew, despite contradictory findings, that the rumen micro-organisms supplied amino acids not present in the feed as ingested by utilizing non-protein nitrogen for the purpose. This was definitely established by 1937. Urea in limited amounts was found a useful source of nitrogen for protein formation in calf and lamb growth, and in milk production by dairy cattle. These results came just in time to find practical application in the use of urea as a component of cattle rations during World War II. Later studies showed that all the amino acids essential for rat growth could be synthesized in the rumen of lambs. This resulted in protein growth, though not at a normal rate. Other amides and ammonium salts were also found useful for protein synthesis in the rumen. These studies have stimulated a very active current research program on the dietary and other factors influencing vitamin as well as protein formation in the rumen. One may expect that other nitrogen compounds besides urea, and in less limited amounts, may find a real place in the rations of ruminants. New problems as to definitions, standards and control will arise accordingly.

Since 1915 there has been a large development in our knowledge as to the number of mineral elements which are essential for body function and as to what ones are of practical importance in the sense that they may be deficient in otherwise good rations unless special attention is given to them. These developments have in turn been reflected in the activities of the A.O.A.C., as the published regulations show. It is to be expected that

future activities may be markedly affected by studies now in progress with respect to the trace elements, particularly as regards the justification for including them in mineral supplements to rations of farm animals.

Supplementary iodine is needed for various species in a rather wide area. There also are area needs for cobalt for ruminants. One or two areas of copper deficiency have also been established for these species. There are special physiological and practical reasons why supplemental manganese is called for in certain poultry rations.

Experiments have been reported in which benefit has been noted from adding mixtures of iron, copper, cobalt, manganese, and zinc to practical hog rations, and contrary findings have also been reported. This may mean that certain rations may need supplementation and others not. The point to be emphasized, however, is that evidence of benefit from a mixture of four or five provides no proof that all are needed. The effect may have been due to one alone. I have cited these examples of the status of present knowledge with respect to trace elements, to illustrate the fact that much more controlled animal research is needed, accompanied by additional and more reliable data on feed composition, before the extent of the real need for supplementary trace elements becomes established. The present situation favors the widespread promotion of the use of complex mineral mixtures, at least as an insurance measure, without any adequate basis. This situation is not to the liking of the nutrition scientist, nor, I presume, to the control official either.

With respect to developments in the field of vitamins, the recent definitions, standards, and other regulations which have been set forth, and the activities in revising and testing assay procedures, clearly show that this impact is being felt and dealt with. The same is true as regards the related developments with respect to antibiotics. The A.O.A.C. and the A.F.C.O. thus have performed an important service to both the livestock and feed industries.

The present nutrition research dealing with amino acids, trace elements, vitamins, and antibiotics is naturally creating a widespread interest among the chemical industry regarding larger markets for its products, and among stockmen eager for further improvements in their rations. The present situation with respect to the new discoveries and their exploitation particularly emphasizes the need for efforts to keep feeding practices sound in terms of established facts. Here the stockmen and the control official, and the feed manufacturer also, as well as the nutrition scientist, have a common interest. Unfortunately, the short-term interests of some producers of special products and others who are looking primarily for a new sales appeal, aided at times by over-enthusiastic investigators, are promoting feeding practices which are unjustifiable in terms of present knowledge. This is not a new development. Over the years I have seen phosphorus, vitamin D, vitamin A, vitamin E, and trace elements pro-

moted for the prevention and cure of abortion and related troubles, and in some cases incorporated into mixed feeds. One promotion died as the lack of benefit became evident, only to be succeeded by another. But the stockmen paid the bill.

Today the increasing tempo of nutrition discoveries, and of commercial developments stemming from them, is creating increased pressure for practical use before the value of a given discovery or development is definitely established. This is occurring in the human as well as the animal field. The situation emphasizes the importance of following up more closely the nutrition discoveries with a thorough assessment of their practical significance and the dissemination of the facts to all concerned. Nutrition science requires the work of men who dedicate their efforts to basic discoveries without direct regard to practical application. The advancement of the over-all field of nutrition, however, equally needs the same scientific and objective consideration of the practical applications of the discoveries made, in terms of a given species and function and of the feed supply concerned. I believe that nutrition scientists, control officials, and the feed industry all have a part to play in this objective.

In bringing this talk to a close, I should like to emphasize that the new developments resulting from the discoveries of nutrition scientists and the new problems thus presented to regulatory officials indicate the desirability of close co-operation between the two groups on many of the problems involved. During the past several years such informal co-operation between the Food and Drug Administration and the Food and Nutrition Board of the National Research Council, on various occasions, has been mutually beneficial and also of service to both the food industry and consumer. There has also been a useful exchange of information, I believe, between feed control officials and various other groups of the Council, notably the Committee on Animal Nutrition and the Committee on Feed Composition. I have the feeling that by taking fuller advantage of these opportunities for co-operation, the increasing impact of nutrition developments on the activities of your Association can be met even more effectively, in terms of the interests of all concerned.

ORDER OF PUBLICATION

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

Third Day

WEDNESDAY—MORNING SESSION

REPORT OF THE EDITORIAL BOARD

K. L. MILSTEAD, *Chairman*

Since our report of last year the sale of the Book of Methods has continued but at a somewhat slower rate. We expect a continuing demand for the book, because the 8th revision is not due until 1955.

At present there is no committee on Revision of Methods, but in anticipation of the 8th edition a committee should be appointed during the coming year so that work on that edition can be started promptly.

REPORT OF THE EDITORIAL COMMITTEE OF THE *JOURNAL*

PAUL A. CLIFFORD, *Editor and Chairman*

Your editor is glad to report that during the year (September to September) the number of subscriptions to the *Journal* rose from 1851 to 1957—a gratifying increase of 106 subscriptions. The increased revenue will help greatly in defraying the printing costs of the *Journal.*

Besides the usual record of proceedings and referee reports there will be 47 contributed papers and notes—somewhat more than our usual number. Many of them are of outstanding excellence. There will be 31 book reviews. Referees and contributors are again urged to make their reports and papers as concise as possible.

It may be of interest to note that our *Journal* ranked 13th among the 28 top ranking periodicals of the world in the field of analytical chemistry. This was for the year 1950, and ranking was based only upon the number of abstracts appearing in the Analytical Section of *Chemical Abstracts.** Many more appear under the topic Foods, and elsewhere. The *Journal* is one of the world's leading scientific periodicals and we can foresee a steady gain in its prestige.

* *Science,* **115,** 555 (May 1952).

REPORT OF COMMITTEE TO CONFER WITH AMERICAN PUBLIC HEALTH ASSOCIATION ON STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS

Your Chairman is pleased to report that the manuscript for the Tenth Edition of *Standard Methods for the Examination of Dairy Products* will be submitted at the Annual Meeting of the American Public Health Association at Cleveland on October 20. Among the noteworthy changes are the following:

1. Milk-free plating media for the agar plate method, consisting either of a Bacto brand or of a BBL brand, will be substituted for the current tryptone glucose meat extract plating medium to which 1 per cent of skim milk is added. The effective date for using the new media will coincide essentially with the publication of the new edition.

2. Systematic studies of 6 staining procedures for the determination of the bacterial content of milk and cream by the direct microscopic method have been completed. Average bacterial counts by three of the 6 stains were essentially identical. Among these 3 is the aniline oil-methylene blue stain of William R. North and M. Thomas Bartram of the Food and Drug Administration. The other two are the acid- and water-free stain of B. S. Levine and L. A. Black of the U. S. Public Health Service at Cincinnati and the polychrome-methylene blue stain of C. A. Anderson of the Department of Public Health, Rockford, Illinois.

3. When using a capillary pipet, as originally proposed by Breed for depositing 0.01 ml. of milk on a micro slide in the direct microscopic method, the operations proceed somewhat slowly. About 25 years ago the dairy industry adopted the use of specially calibrated wire loops for the transfer of the 0.01 ml. portions in order to speed up operation. The new edition of Methods will recognize an all metal syringe, with semi-automatic actuated piston, for delivering the test portions. The syringe can be operated essentially as rapidly as the loop and assures equally as uniform measurements as can be made with the pipet.

4. Directions for applying the coliform test as an index of recontamination of dairy products following pasteurization have been clarified.

5. Directions for the milk ring test for detecting brucellosis infections are included.

6. Because of difficulties encountered with sampling milk in large storage tanks and vats, directions are included to guide both the equipment manufacturers in their construction and the dairy industry in their use so as to assure proper mixing of the contents before taking samples.

7. Improvements have been made by Curtis R. Joiner with the fine standard sediment mixture for comparison with test samples from retail milk supplies. The use of the fine standard sediment mixture will supplement the use of the coarse standard sediment mixture.

8. A paragraph is included to govern gross misinterpretations on the activity of bacterial growth inhibitors in dairy products, such as (1) residual chemical sanitizers, (2) residual sulfa drugs, (3) residual antibiotics, (4) bacteriophages, and (5) other unknown and unidentified agents. Some cheese manufacturers have attributed delayed starter action to traces of quaternary ammonium compounds and others to traces of antibiotics. Users of reduction type methods for determining bacterial densities have observed differences in reduction time intervals in samples to which antibiotics in usual therapeutic (injection) doses are added, as compared with untreated duplicate samples. Despite these observations, it seems inadvisable to draw hasty conclusions as to the exact cause for a delayed acidity development, a prolonged reduction time, an unusually low agar plate count, etc., until assured by objective tests that all other possible inhibitors are inactive.

HENRY A. LEPPER
WILLIAM HORWITZ
A. H. ROBERTSON, *Chairman*

REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES

WM. F. REINDOLLAR, *Chairman*

The necessity of holding the annual meeting at an unusually early date this year has the marked disadvantage of shortening the time available for collaborative studies and the completion of referee reports. In spite of this handicap over 150 reports were received in time for inclusion in the published program, a fact which reflects the interest and industry of referees and collaborators alike.

While the activities of the chairman are principally those of coordination and stimulation of the program, the major tasks—those of reviewing, studying, appraising, and making appropriate recommendations on the numerous studies submitted—fall upon the four constituent subcommittees; hence their presentations constitute the sum and substance of the report.

The need for additional referees, associate referees, and collaborators is both a continuing and expanding one; it is therefore strongly urged that all members volunteer for participation in these important activities of our Association.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES

E. L. GRIFFIN, Production and Marketing Administration, Department of Agriculture, Washington 25, D. C., *Chairman;* J. B. SMITH; and C. V. MARSHALL

FEEDING STUFFS

It is recommended—

(1) That study of tankage (hide, hoof, horn, and hair content) be discontinued.

(2) That work on the following be continued:

(a) Fat in fish meal.
(b) Crude fat or ether extract.
(c) Mineral constituents of mixed feeds.
(d) Drugs in feeds.
(e) Crude protein in feeding stuffs.
(f) Ash in feeding stuffs.
(g) Milk by-products in mixed feeds.
(h) Microscopic examination.

(3) That the method for Enheptin® (2-amino-5-nitrothiazole), with alterations as outlined by the Associate Referee, be further studied collaboratively.

(4) That collaborative studies of a method for nitrophenide (m,m'-dinitrodiphenyldisulfide) be continued.

(5) That the method for sulfaquinoxaline, **32.191–32.193,** adopted first action in 1949, be made official.

(5) That the method for the determination of cobalt in mineral feeds, adopted first action last year, be made official.

(7) That the method for crude fat in baked dog food as recommended by the Associate Referee be adopted first action.

FERTILIZERS

It is recommended—

(1) That further colaborative work on the Shuey method for nitrogen be discontinued since the results obtained by it are not significantly better than those obtained by the official method.

(2) That the phrase "(0.1–0.3 g $CuSO_4 \cdot 5H_2O$ may also be added)" be inserted in **2.26** following "Anhyd. Na_2SO_4," and the method adopted as official.

(3) That study of sampling and sample preparation be continued.

(4) That consideration be given to the study of methods of sampling and analysis of liquid fertilizer mixtures.

(5) That the first action modified Perrin method be adopted as an official alternate method for potash in fertilizers.

(6) That additional studies be made toward adapting the flame photometer for use in the determination of potash in fertilizers.

(7) That work on carbonate carbon or calcium carbonate equivalent and acid insoluble ash in fertilizers be continued.

(8) That the Virginia modified method for boron in mixed fertilizer be adopted first action and the work be continued.

(9) That work on magnesium in coarse particles in fertilizers be continued.

(10) That the determination of citrate-insoluble P_2O_5 in calcium metaphosphate on samples ground to pass the 35-mesh sieve, with continuous agitation during the citrate digestion, be made official.

(11) That further study be made of methods for direct determination of available P_2O_5 in fertilizers.

(12) That study be made of the use of perchloric acid in preparation of phosphate fertilizer solutions for analysis.

(13) That the parenthetical statement "(Ammoniacal N×2=total N in NH_4NO_3)" be deleted from **2.28,** official.

(14) That all Referees continue their work during the coming year; that an additional Associate Referee be appointed to study sampling and sample preparation of ammoniacal solutions, and that the Referees on various "Trace" elements each give attention to analysis of products of low solubility which contain their elements.

ECONOMIC POISONS

It is recommended—

(1) That the changes in the methods for pyrethrins, **5.111, 5.114,** that were adopted as first action at the 1951 meeting be dropped; that this action take effect immediately and that notice of it be sent to the mailing list of *Changes in Methods.*

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

(3) That study of the methods for piperonyl butoxide be continued.

(4) That the revised Elmore method for the determination of thiocyanate nitrogen in fly sprays, adopted first action in 1950, be made official and that the subject be dropped.

(5) That the hydrogenation and the ethylenediamine methods of analysis for technical allethrin be further investigated and studied collaboratively.

(6) That study of methods for isopropyl N-phenylcarbamate be continued.

(7) That methods be studied for determination of physical properties of economic poisons, especially particle size and dispersibility in aqueous and dry formulations.

(8) That a collaborative study of methods for warfarin concentrates be made.

(9) That the study of methods for analysis of low percentage warfarin baits be continued, and collaborative studies undertaken if advisable.

(10) That study of the methods for benzene hexachloride be continued.

(11) That study of methods for rotenone be continued.

(12) That the method for determination of potassium cyanate in herbicides, adopted first action last year, be made official.

(13) That the method for the determination of total chlorine in esters of 2,4-D and 2,4,5-T in liquid herbicides by the Parr bomb-boric acid procedure be adopted first action.

(14) That method 369 (23A Revised) (*This Journal*, **33**, 767, (1950)) for the determination of ester type compounds of 2,4-D and 2,4,5-T in herbicides be further studied.

(15) That the application of the partition chromatographic procedure for determination of 2,4-D and 2,4,5-T in mixtures of these herbicides be studied.

(16) That the method for parathion, adopted first action last year, be modified to use the potentiometric end-point technique described by the Associate Referee and further studied collaboratively.

(17) That the investigation of the analysis of parathion emulsifiable concentrate be continued.

(18) That a referee be appointed to study determination of volatility of the ester forms of hormone-type herbicides by biological methods.

(19) That the work of the other Associate Referees on economic poisons be continued.

(20) That a referee be appointed to study the analysis of systemic insecticides.

DISINFECTANTS

It is recommended—

(1) That the "use-dilution" methods for evaluating disinfectants as described in the paper entitled "Use-dilution Confirmation Tests for Results Secured by Phenol Coefficient Methods" by Stuart, Ortenzio and Friedl be adopted first action.

(2) That the work of the Associate Referee on media ingredients be continued.

(3) That the work on the official method for fungicides be continued as suggested by the Referee.

PLANTS

It is recommended—

(1) That the nitroso-R-salt method used in the 1952 collaborative study of the determination of cobalt in plants be adopted first action.

(2) That the nitroso-cresol method used in 1949 and 1951 collaborative studies of the determination of cobalt in plants be adopted first action.

(3) That the sodium diethyldithiocarbamate method for copper in plants be adopted first action.

(4) That the study of methods for the determination of sodium in plants be continued especially with respect to:

(a) Comparison of the values for sodium by the use of the flame photometric and the A.O.A.C. magnesium uranyl acetate procedures in the analysis of a variety of plant materials.

(b) Use of the A.O.A.C. method in the analysis of plant materials which contain only small amounts of sodium.

(c) Possible interferences of various ions that commonly occur in plant materials.

(5) That the modified procedure described by the Associate Referee for the determination of small amounts of starch in plant materials be submitted to collaborative study.

(6) That the micro method for dextrose recommended by the Associate Referee be adopted first action.

(7) That the collaborative study of the ion-exchange method of clarifying solutions for the determination of sugar be continued.

(8) That the study of methods for determining zinc in plant materials be continued.

(9) That the work of the other Associate Referees on methods for plant materials be continued.

(10) That an Associate Referee be appointed to study flame photometric procedures for the determination of potassium in plants.

SOILS AND LIMING MATERIALS

It is recommended—

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

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

(3) That the utilization of carmin as an indicator in the determination of boron content of soils be studied further, and that p-nitro benzenazo-1,8-dihydroxy naphthalene-3,6-disulphonic acid, or "chromotrope-B" be studied as a suitable reagent in that determination.

(4) That the neutral calcium acetate method for the replacement and the determination of exchangeable hydrogen of soils be adopted as official.

(5) That the survey and comparison of methods for the determination of phosphorus (a) that fraction in "available" state and (b) the proportion of organic-inorganic forms therein, be continued (*This Journal*, **30**, 43 (1947)).

(6) That the survey and comparison of methods for the determination of exchangeable potassium in soils (*This Journal*, **30**, 44 (1947)) be continued, and that a detailed procedure be prepared and studied collaboratively.

(7) That the Associate Refereeship on Exchangeable Calcium and Magnesium be continued.

STANDARD SOLUTIONS

It is recommended—

(1) That the study of standard sodium thiosulfate solutions be continued.

(2) That the Constant Boiling Method for standardizing hydrochloric acid, **39.11,** be modified as recommended by the Associate Referee and adopted first action.

VITAMINS*

It is recommended—

(1) That the method for Vitamin A in mixed feeds be further studied as suggested by the Associate Referee.

(2) That the method for determining Vitamin A in oleomargarine be further studied.

(3) That the study of methods for thiamine in enriched cereal products be continued.

(4) That further work be undertaken to study a suitable chemical method for Vitamin B_6.

(5) That the sulfanilic acid method for nicotinic acid be made official.

(6) That the method for assay of Vitamin B_{12} feed supplements ranging from approximately 1.0 mg to 10 mg of Vitamin B_{12} per pound, as described by the Associate Referee, be made first action.

(7) That the study of the microbiological assay for Vitamin B_{12} be continued as suggested by the Associate Referee.

(8) That work on other vitamins be continued.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES

FRANK H. WILEY (Division of Pharmaceutical Chemistry, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Chairman;* H. J. FISHER; and G. ROBERT CLARK

RADIOACTIVITY

The committee recommends that the subject be continued.

SPECTROGRAPHIC METHODS

The committee recommends the continuance of the subject and, in the interest of clarity, recommends that the title be changed to Emission Spectrography.

VEGETABLE DRUGS AND THEIR DERIVATIVES

It is recommended—

(1) That the method for the determination of rutin as revised by the subcommittee so that the calculation of the rutin content is based on the values obtained from the standard, be adopted, first action and the subject be discontinued.

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* Note change of title from "Vitamins" to "Nutritional Adjuncts," *This Journal* 36, 5 (1953).

(2) That the work on a method for quinine and strychnine be continued according to the suggestions of the Referee and that its application to elixir of iron, quinine, and strychnine be studied.

(3) That the study on aminopyrine, ephedrine, and phenobarbital be continued.

SYNTHETIC DRUGS

It is recommended—

(1) That the study of methods for sulfanilamide derivatives be continued with investigation of recently advocated titrimetric procedures in organic solvents.

(2) That the spectrophotometric method for isonicotinyl hydrazide be submitted to collaborative study.

(3) That the study of methods for amobarbital sodium and secobarbital sodium be continued for the purpose of attempting to apply a background correction.

(4) That the following topics be continued:

Methylene Blue
Propadrine
Spectrophotometric methods
Synthetic estrogens
Di-phenhydramine and tripelennamine hydrochlorides
Amphetamines.

MISCELLANEOUS DRUGS

It is recommended—

(1) That the method for propylene glycol be adopted, first action.

(2) That the subject of alkali metals be reassigned and the application of the flame photometer be investigated.

(3) That the following topics be continued:

Microscopic tests for alkaloids and synthetics
Mercury compounds
Organic iodides and separation of halogens
Preservatives and bacteriostatic agents in ampul solutions.

COSMETICS

It is recommended—

(1) That the study of sun tan preparations be discontinued.

(2) That the following topics be continued:

Deodorants and anti-perspirants
Cold permanent wave preparations
Cosmetic creams
Mascara, eyebrow pencil, and eye shadow
Hair dyes and rinses.

COAL TAR COLORS

It is recommended—

(1) That subsidiary dyes in FD&C colors—determination of lower sulfonated dyes in FD&C Blue No. 1 be submitted to collaborative study and the topic continued.

(2) That collaborative study be undertaken on paper chromatography of coal-tar colors and the topic be continued.

(3) That no action be taken on the proposed method for subsidiary dyes in D&C Red No. 35 until the Associate Referee has had an opportunity to compare it with spectrophotometric methods.

(4) That the following topics be continued:

Boiling range of amines derived from coal-tar colors
Inorganic salts in coal-tar colors
Ether extracts in coal-tar colors
Halogens in halogenated fluoresceins
Identification of coal-tar colors
Volatile amine intermediates in coal-tar colors
Unsulfonated phenolic intermediates in coal-tar colors
Non-volatile unsulfonated amine intermediates in coal-tar colors
Intermediates derived from phthalic acid
Lakes and pigments
Spectrophotometric testing of coal-tar colors
Determination of arsenic and antimony in coal-tar colors
Determination of heavy metals in coal-tar colors
Sulfonated phenolic intermediates in coal-tar colors
Intermediates in triphenylmethane dyes

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES

A. H. ROBERTSON (State Food Laboratory, Dept. of Agriculture and Markets, Albany, New York), *Chairman;* SAM ALFEND; FRANK A. VORHES, JR.

GENERAL RECOMMENDATIONS

The Committee recommends that each General Referee study all methods now in first action status with a view to recommending appropriate action, with collaborative study, if necessary, as soon as possible.

PROCESSED VEGETABLE PRODUCTS

It is recommended—

(1) That work on methods for determining quality factors in canned and frozen fruits and vegetables, determination of moisture in dried vegetables, and enzymatic action in frozen vegetables be continued.

(2) That collaborative study of the rapid method for the determination of residual catalase activity in frozen vegetables be continued.

(3) That the method for the determination of acetaldehyde as an index of quality deterioration in frozen vegetables be submitted to further collaborative study.

(4) That the ascorbic acid method for peroxidase be further studied.

COFFEE AND TEA

It is recommended—

(1) That the study of methods for determining chlorogenic acid be continued and include collaborative study.

(2) That study on the determination of moisture in coffee and tea be continued, and include preparation of sample.

DAIRY PRODUCTS

It is recommended—

(1) That the method described in the report of the Associate Referee be adopted as a procedure for the preparation of samples of creamed cottage cheese.

(2) That further work be done on methods for preparation of samples of frozen desserts which contain insoluble material, such as fruits, nuts, etc.

(3) That further work be done on methods for determination of sucrose and acidity of ice cream and frozen desserts.

(4) That further collaborative work be done with the mechanical shaking method for the preparation of butter samples.

(5) That work be continued with the Babcock method for determination of fat in homogenized milk.

(6) That studies be made on substitutes for sulfuric acid in the Babcock method for fat in milk and cream.

(7) That the official methods for acidity of milk, **15.4,** and cheese, **15.129,** be modified by permitting expression of acidity as ml 0.1 *N* NaOH for 100 g sample.

(8) That methods **15.40–.46** and **15.67,** residual phosphatase in milk and cream, be adopted as official.

(9) That work on detection and estimation of foreign fats in dairy products be continued.

EGGS AND EGG PRODUCTS

It is recommended—

(1) That the method for glycerol in eggs, **16.27–.28,** be modified as described in the Associate Referee's report, applicable to eggs with or without added sugar, be adopted as first action, and that work on the subject be closed.

(2) That work on the determination of ammonia nitrogen in eggs be continued along the lines indicated by the Associate Referee in 1951.

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL INDICES)

It is recommended—

(1) That study on galacturonic acid as a measure of decomposition in apples and spinach be discontinued.

(2) That studies of methods for intermediate polygalacturonides as an index of decomposition in fruits be discontinued.

(3) That gluconic acid be studied as an index of decomposition in fruits.

(4) That search be made for other indices of decomposition in fruits.

(5) That studies to determine the correlation between rot and succinic acid in tomatoes be expanded to include (a) study of bacterial rots, and (b) storage experiments with processed products made from sound tomatoes.

(6) That study of histamine as an index of decomposition in fish be continued.

(7) That search for suitable chemical indices of chemical decomposition in shellfish be continued.

(8) That studies of uric acid as an index of filth in nuts, cereals, and eggs be continued, and that study of uric acid in fruits be discontinued.

(9) That search for chemical indices of fecal matter in foods be continued.

(10) That study of chemical methods for detection of decomposition in nuts be continued.

GELATIN DESSERT PREPARATIONS AND MIXES

It is recommended—

(1) That work on methods for sucrose and dextrose, **21.13, 21.14,** and **21.15** be continued.

(2) That the first action methods for jelly strength, **21.6** and **21.12,** be made official.

FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the method for determination of total solids in fish and other marine products, *This Journal,* **35,** 216 (1952), be compared with a forced draft oven method and collaborative study be undertaken.

(2) That the first action method for total solids in oysters (**18.1** and **18.4**) be modified as described by the Associate Referee and adopted, first action.

(3) That the rapid method for ether extract, described by the Associate Referee, be subjected to collaborative study.

(4) That the use of chemical and viscosimetric methods for determination of solids in oysters be investigated.

METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

It is recommended—

(1) That study of methods for determining copper and zinc be continued.

(2) That the first action method for mercury, *This Journal* **35,** 80 (1952), be adopted as official.

(3) That the effect of canning and other processes on the decomposition of the newer pesticides be further studied with respect to the nature of possible decomposition products and their effects on methods of analysis.

(4) That studies on the determination of DDT be continued with at-

tention to the Stiff-Castillo colorimetric method as an alternative procedure.

(5) That the qualitative and quantitative methods for sodium fluoroacetate (1080) be further studied.

(6) That studies on the differentiation between methoxychlor and DDT in plant and animal products be continued, and collaborative work on the method for methoxychlor be initiated.

(7) That collaborative studies be made on the methods for the determination of benzene hexachloride and its beta isomer.

(8) That collaborative study be made of modifications designed to eliminate the effect of silica in the determination of fluorine.

(9) That methods for the determination of trace amounts of chlordane, heptachlor, aldrin, dieldrin, toxaphene and other chlorinated insecticides be studied.

(10) That methods for determination of trace amounts of phosphorus-containing insecticides, including parathion, be studied.

(11) That flame photometric and chemical methods for the determination of sodium in foods be developed.

GUMS IN FOOD

It is recommended—

(1) That the method for the detection of gums in catsup and related products be studied collaboratively.

(2) That studies be made on the detection of algin in cheese.

(3) That study of the methods for detection of gums and algin in chocolate milk be extended to other dairy drinks and fountain materials.

(4) That work be continued on the detection of gums in frozen desserts.

(5) That work be continued on the detection of the other emulsifying agents which are permitted by the Federal Standard for french and salad dressing.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That work be continued on the proposed method for starch in meat products.

(2) That work be continued on chemical and serological methods for detection of horse meat in ground meat.

(3) That work be continued on the proposed method for creatin in meat products.

(4) That the method for moisture in meat, **23.2,** be modified by dropping the reference to **22.7** and inserting the parenthetical statement after **22.3:** "(Not suitable for high fat products such as pork sausage.)"

(5) That the air drying methods for moisture in meat described by the Associate Referee be adopted as first action.

(6) That the method for crude fat in meats using petroleum ether as described in the Associate Referee's report be subjected to collaborative study.

NUTS AND NUT PRODUCTS

It is recommended—

(1) That methods for moisture, crude fat, crude protein, crude fiber, ash, reducing sugar and salt be further studied.

(2) That sorting methods for moisture and fat be studied.

(3) That methods for added starch and other additives in peanut butter be studied.

(4) That study of methods for added propylene glycol, glycerine, and sorbitol in shredded coconut be continued.

MICROBIOLOGICAL METHODS

It is recommended—

(1) That collaborative studies be made of the first action methods for sugar and canned vegetables.

(2) That the first action method for eggs and egg products be amended as recommended by the Referee and adopted first action and that the modified method be studied collaboratively.

(3) That work be continued on methods for frozen fruits and vegetables, canned fruits and other canned acid foods, fish and fishery products, nuts and nut products, and canned meats.

(4) That this chapter be expanded by the inclusion of tested methods applicable to other products.

MICROCHEMICAL METHODS

It is recommended—

(1) That the Carius method for bromine and chlorine described in the Associate Referee's report be adopted as first action.

(2) That studies be continued on the catalytic combustion method for bromine and chlorine.

(3) That the Carius and catalytic combustion methods described in the Associate Referee's report, with titrimetric determination of sulfates, be adopted as first action.

(4) That further collaborative work be done on the gravimetric method for determining sulfate formed in the Carius and catalytic combustion methods.

(5) That further collaborative work be done on the Dumas procedure for nitrogen.

(6) That the micro Kjeldahl method for nitrogen be further tested on compounds containing NO and NO_2 groups and those containing N-N linkages.

OILS, FATS, AND WAXES

It is recommended—

(1) That studies on quantitative methods for peanut oil be continued.

(2) That studies on spectrophotometric methods for analysis of oils be continued.

(3) That the first action method for determination of propyl gallate be further studied.

(4) That collaborative studies on methods for determination of butylated hydroxyanisole and nordihydroguaiaretic acid be conducted.

SPICES AND OTHER CONDIMENTS

It is recommended—

(1) That studies of methods for the detection of caramel in vinegar be continued.

(2) That studies on the determination of tartrates in vinegar be continued.

(3) That methods for the determination of free mineral acids in vinegar be studied.

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

(5) That study of methods for the determination of ash and sugar in prepared mustards be continued.

(6) That studies of methods for the determination of pungent principles in prepared mustard and mustard flour be conducted.

(7) That study of the official method for total nitrogen in mayonnaise and salad dressing be conducted.

(8) That the methods for the detection of seeds and stems in chili be further studied and submitted to collaborative trial.

(9) That studies on the preparation of samples of french dressing in large size containers be continued.

(10) That study of the method for volatile oil in spices be continued.

ENZYMES

It is recommended—

(1) That an Associate Referee be appointed to develop enzymic methods for small quantities of insecticides.

(2) That work on hydrocyanic glucosides be discontinued.

(3) That methods for testing the activity of the various enzymes used in analytical procedures be studied for the purpose of inclusion in the chapter on enzymes.

(4) That the first action urease-bromthymol blue test paper method for urea be further studied preparatory to final action.

(5) That study of methods for the preparation of enzymes which may not be available commercially be discontinued.

REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES

J. WALTER SALE (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Chairman;* FLOYD ROBERTS; and KENNETH L. MILSTEAD

MALT BEVERAGES, BREWING MATERIALS AND ALLIED PRODUCTS

It is recommended—

(1) That the method for the determination of yeast-fermentable sugar in beer by A. P. Mathers and J. E. Beck be studied collaboratively.

(2) That work on methods for turbidity and color in beer and for the degassing of beer be discontinued.

(3) That the study of the wet-ash orthophenanthroline method for iron be continued and after completion be submitted to collaborative study.

(4) That the direct, non-ash orthophenanthroline procedure for iron adopted first action last year, be further studied by the Associate Referee.

(5) That collaborative studies on copper be postponed.

(6) That the methods of Stone and of Kuznetsov and Bender for the determination of tin be studied.

(7) That the collaborative work on tin be postponed until work on iron and copper has been completed.

(8) That collaborative studies of methods for the determination of moisture, total nitrogen, and P_2O_5 in yeast be continued.

(9) That collaborative studies of methods for the determination of ash, ether extract, crude fiber, and crude carbohydrate in yeast be postponed until the studies on moisture, total nitrogen, and P_2O_5 are completed.

WINES

It is recommended—

(1) That collaborative work be continued on the method for the determination of non-volatile acids in wines by paper chromatography.

(2) That collaborative work be continued on the first action spectrophotometric method for determining phosphates in wines and spirits.

(3) That collaborative work be continued on the spectrophotometric method for tannin in wines and whiskies.

(4) That work be conducted on the determination of color in wines.

DISTILLED LIQUORS

It is recommended—

(1) That the official method, **9.29,** for methanol by the immersion refractometer method, be studied in the light of the findings of Beyer and Reeves, *This Journal,* **28,** 800 (1945).

(2) That study be continued on the methods for methanol in distilled liquors and drugs with a view to their correlation.

(3) That the investigation of methods for the determination of higher alcohols in distilled spirits by chromatography be continued.

CORDIALS AND LIQUEURS

It is recommended—

(1) That the method for citric acid in fruits and fruit products, *This Journal*, **34**, 74 (1951), be studied collaboratively on cordials and liqueurs.

CACAO PRODUCTS

It is recommended—

(1) That the work on methods for the determination of maltose in cacao products be continued.

(2) That work on the determination of lactose in cacao products containing other reducing sugars be continued.

(3) That the revised method for lecithin in cacao products, studied collaboratively this year, be made first action.

(4) That the study of characteristic cacao constituents such as cacao red, theobromine, etc., be continued.

(5) That work on a hydrolytic colorimetric method for pectic acid in cacao products and work on the determination of pectic acid in cacao products containing milk be continued.

CEREAL FOODS

It is recommended—

(1) That the study on the determination of starch in raw and cooked cereals be continued.

(2) That the method for sugar in baked products be further studied as recommended by the Associate Referee (essentially **13.30–13.32** inclusive on flour).

(3) That the method for the determination of lactose in bread, *This Journal*, **35**, 697 (1952) be further studied.

(4) That the study of methods on soy bean flour for moisture, ash, nitrogen, crude fiber and oil be continued.

(5) That the method referred to in *This Journal*, **25**, 83–84 (1942) for the determination of unsaponifiable matter and sterols in noodles, be studied for its application to bakery products containing eggs.

(6) That the first action method for water-soluble protein-nitrogen precipitable by 40% alcohol (albumen) in cereal products, *This Journal*, **35**, 75 (1952) be adopted as official and method **13.34** be deleted, final action.

(7) That the study on the determination of bromates in flour be continued.

(8) That the method in *This Journal* **34**, 64–68 (1951) under III "Apparatus" ((b) p. 65) be changed as recommended by the Associate Referee and the revised method adopted as official and that this study be discontinued.

(9) That the official method for lipoid sec. **13.35** and lipoid P_2O_5 sec. **13.36** be deleted.

(10) That the procedure for lipoids and procedure I for lipoid P_2O_5 re-

ported this year by the Referee be adopted first action and the study continued.

(11) That the method for choline in noodles reported by the Referee this year be further studied.

(12) That methods for moisture be studied.

BAKING POWDER

It is recommended—

(1) That the work as reported in *This Journal* **34,** 60 (1951) on the neutralizing value of phosphate be continued.

EXTRANEOUS MATERIALS IN FOODS AND DRUGS

It is recommended—

(1) That the changes proposed by the Associate Referee in the method for the determination of sediment in milk be adopted, first action except with respect to the description of the filtering apparatus.

(2) That work be continued on extraneous materials in vegetable products.

(3) That the revised method proposed by the Associate Referee for the determination of light filth in peanut butter be adopted as first action and that the present method **35.26** be dropped.

(4) That the revised method proposed by the Associate Referee for water insoluble inorganic residue (WIIR) and excreta in peanut butter be adopted as first action and that the present method **35.25** be dropped.

(5) That the methods for the determination of extraneous material in ground cinnamon and turmeric, ground onion powder and ground black and white pepper published in *This Journal,* **35,** 328–330 (1951) be adopted as first action and the present methods **35.83, 35.84, 35.85** and **35.86** be dropped.

(6) That the revised method for the determination of extraneous material in ground capsicums described in the Associate Referee's report be studied collaboratively.

(7) That the study of the method for the identification of manure fragments in dairy products be continued.

(8) That the method proposed by the Associate Referee for the determination of internal insect infestation of wheat be studied collaboratively.

(9) That the revised pancreatin digestion method for insect fragments and rodent hairs in flour proposed by the Associate Referee be studied collaboratively.

(10) That the procedure for the preparation of isopropyl alcohol-gasoline mixture proposed by the Associate Referee be added to the section on special techniques **35.4.**

(11) That an Associate Referee on methods for identification of insect contaminants in food and drug products be appointed.

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That the "First Action Photometric Method for Vanillin," *This Journal*, **34**, 72 (1951) and **35**, 77 (1952), be made official for

(a) Vanilla,
(b) Vanilla containing added Vanillin and/or Coumarin, and
(c) Imitation Vanilla.

(2) That the "First Action Photometric Method for Coumarin," *This Journal*, **34**, 73 (1951) and **35**, 77 (1952), be made official for

(a) Vanilla,
(b) Vanilla containing added Coumarin and/or Vanillin, and
(c) Imitation Vanilla.

(3) That studies be continued on method **19.4** and **19.5.**

(4) That the official colorimetric method for vanillin **19.6** and **19.7** (p. 306) be deleted, final action.

(5) That collaborative studies be continued on the following:

(a) First action method for isopropyl alcohol in lemon and orange flavors, *This Journal*, **35**, 77 (1952).
(b) First action method for essential oil in emulsions, *This Journal*, **35**, 78 (1952).
(c) First action method for essential oil in citrus juices and other beverages, *This Journal*, **35**, 79 (1952).
(d) Beta-ionone when small amounts are present.
(e) Propylene glycol in vanilla extracts, *This Journal*, **33**, 103 (1950).

FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That the official method for citric acid, *This Journal*, **34**, 75 (1951), be revised by deleting the sentences "Dilute 20 ml of the isolated acid solution with 50 ml of water and boil a few minutes to expel H_2S. Cool and titrate with 0.1 N NaOH using phenolphthalein indicator," and that the revised method be made official (by suspension of rules).

(2) That the revision of the first action method for tartaric acid proposed by the Associate Referee be adopted as official.

(3) That the method for *l*-malic acid, applicable in the absence of iso citric acid, as given in this year's report be adopted as a first action alternate method.

(4) That the study of methods for the determination of fruit acids be continued.

(5) That the study of methods for the examination of frozen fruits for fruit, sugar and water content be continued.

(6) That the study of methods for the determination of fill of container for frozen fruits be continued.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

It is recommended—

(1) That the colorimetric method for the detection of P-4000 described in the report of the Associate Referee be submitted to collaborative study.

(2) That the colorimetric method for the determination of P-4000 described in the report of the Associate Referee be submitted to collaborative study.

(3) That study of methods for the determination and identification of cyclamate of sodium and of calcium be continued.

(4) That further work be done on the determination of monochloracetic acid in fruit juices other than orange juice.

(5) That collaborative study of the method for monochloracetic acid in beverage bases containing halogenated weighting oils, *This Journal*, **34**, 345 (1951), be continued.

(6) That the method "Quaternary Ammonium Compounds in Milk, Qualitative," as reported by the Associate Referee, be adopted first action.

(7) That the test for purity of the bromphenol blue reagent as described in the Associate Referee's report on Quaternary Ammonium Compounds, be added to **27.34.**

(8) That collaborative study be continued on the reineckate methods for the determination and identification of Quaternary Ammonium Compounds.

(9) That collaborative study be continued on the bromphenol blue methods for Quaternary Ammonium Compounds, *This Journal*, **29**, 318 (1946), **33**, 670 (1950), to include methods for fruit juices, bottled sodas, milk, mayonnaise, pickles and shrimp.

(10) That further study of a spectrophotometric method for the quantitative determination of benzoic acid be made.

(11) That the study of methods for the detection of benzoates and hydroxybenzoates be continued.

(12) That the method for the detection of the fluoride ion by the quenching of aluminum oxinate fluorescence be submitted to collaborative study.

(13) That study of thiourea be discontinued.

(14) That the qualitative test and the spectrophotometric method for dehydroacetic acid in cheese be adopted first action.

(15) That the method for the determination of dimethyldichlorsuccinate reported by Ramsey this year be studied collaboratively.

(16) That Associate Referees be appointed to work on the following subjects:

(a) Hydrogen peroxide in dairy products.
(b) Peracetic acid in fruit juices.
(c) Gallates with 8–12 carbon atoms in oily foods, fish, meat or baked goods.

SUGARS AND SUGAR PRODUCTS

It is recommended—

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

(2) That the study be continued on tables of density of solutions of sugars at various temperatures.

(3) That the study of methods for the detection of adulteration of honey be continued.

(4) That the study of methods for the determination of reducing sugars including those employing chromatographic separation be continued.

(5) That the study of methods, **29.132–29.154,** inclusive, be continued.

(6) That collaborative work be carried out on the volumetric determination of small amounts of reducing sugars by the Lane and Eynon method and by the constant volume modification.

(7) That Zerban and Martin refractive index values for raffinose hydrate solutions be made official.

(8) That the study of the transmittancy of sugar solutions be continued by repeating this year's work on the reproducibility of the Celite filtration method, with a more exact standardization of the procedure used.

(9) That the refractive indices of sucrose solutions in the range 60 to 70 per cent be studied collaboratively.

(10) That collaborative work be initiated on the Somogyi phosphate method, to include comparative studies between the phosphate and carbonate methods.

(11) That collaborative work be conducted on methods for determining total sugars in hydrol.

WATERS, MINERAL AND SALT

It is recommended—

(1) That the first action method for boron in water be studied collaboratively, and that other methods for boron in water be investigated.

(2) That a colorimetric method for phosphates in water be studied.

CHANGES IN OFFICIAL METHODS OF ANALYSIS MADE AT THE SIXTY-SIXTH ANNUAL MEETING, SEPTEMBER 29, 30 AND OCTOBER 1, 1952

The changes recorded in the methods of the Association, as given below, become effective, as provided in Section 8 of the by-laws, on the thirtieth day from the date of publication of this Report, February 15, 1953.

There is appended a list of errata and emendations in the seventh edition of "Official Methods of Analysis, 1950."

1. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or other changes.

2. FERTILIZERS

(1) The statement "(0.1–0.3 g $CuSO_4 \cdot 5H_2O$ may also be added.)" was inserted in the "Gunning Method Modified to Include Nitrogen of Nitrates," **2.26** (p. 13–14) following ". . . or anhyd. Na_2SO_4," and the revised method was adopted as official.

(2) The parenthetical statement "(Ammoniacal N×2=total N in NH_4NO_3.)" in the official "Formaldehyde Titration Method" for ammoniacal nitrogen, **2.28** (p. 14), was deleted, official.

(3) The first action wet digestion method for potash, *This Journal*, **35** 61 (1952), was adopted as official.

(4) The following method for boron in mixed fertilizers was adopted, first action:

Weigh 2.5 g sample into a 250 ml beaker. Add 125 ml H_2O and boil gently for ca 10 min., filter hot thru #40 Whatman paper into 400 ml beaker. Wash solids well with hot H_2O (6 washings) and make vol. to at least 200 ml with distd H_2O. Heat filtrate just to boiling. Add 15 ml 10% $BaCl_2$ to ppt sulfates and phosphates and add powd $Ba(OH)_2$ cautiously with stirring, until just alk. to phenolphthalein, avoiding large excess. Boil in open beaker at least 60 min. to expel NH_3. Longer boiling for samples colored by organic matter is desirable. If necessary, add H_2O to keep vol. to at least 150 ml. Add and stir 1–2 tsp. of Filter-cel or other inert filtering aid, and filter with suction thru packed paper pads into 500 ml Pyrex Erlenmeyer flask. Wash ppt 6 times with hot boiled distd H_2O. (Too large wash volumes are to be avoided; such washings increase the vol. in the flask to the point of dangerous bumping in the next step.) Make filtrate just colorless to phenolphthalein with 6% HCl, add methyl red indicator and make just pink with the acid. Add 5 or 6 boiling stones and a stirring rod, cover with watch glass and boil 5 min. to remove CO_2. Cool in cold water, covered. Wash cover glass, stirrer and sides of flask. Titrate to the yellow of methyl red with standard 0.05 *N* NaOH. Add 20 g d-mannitol and 1 ml or more of 1% phenolphthalein soln, shake and wash down sides. Titrate to pink end point. Run blank in exactly same manner as sample. 1 ml 0.05 *N* NaOH = 0.000540 g B or 0.00477 g borax. Or, (Titer − blank) × factor = lb borax per ton (factor = 3.807 for 0.05 *N* NaOH).

(5) The application of the official "Citrate-Insoluble Phosphoric Acid" method, **2.16** and **2.17** (p. 10), using continuous agitation during digestion to calcium metaphosphate on samples ground to pass a 35 mesh sieve, *This Journal*, **35,** 61 (1952), was adopted as official.

3. SOILS

(1) The first action neutral calcium acetate method for the replacement and the determination of exchangeable hydrogen of soils, *This Journal*, **35,** 62 (1952), was adopted as official.

4. CAUSTIC POISONS

No additions, deletions, or other changes.

5. ECONOMIC POISONS

(1) The *changes in the methods* for Pyrethrin I, **5.111,** and for Pyrethrum Extracts in Mineral Oil, Pyrethrin I, **5.114,** which were adopted first action, *This Journal*, **35,** 64 (1952), items (2) and (4), were dropped. This action was to take effect immediately (October 1, 1952); notice of the change was to be sent to the mailing list of *Changes in Methods*. (Mailing was completed October 11, 1952.)

(2) The first action method for the determination of organic thiocyanate nitrogen in livestock or fly sprays, *This Journal,* **34,** 59 (1951), was adopted as official.

(3) The first action method for the determination of potassium cyanate in herbicides, *This Journal,* **35,** 63 (1952), was adopted as official.

(4) The following method for the determination of total chlorine in esters of 2,4-D and 2,4,5-T in liquid herbicides by the Parr bomb-boric acid procedure was adopted, first action:

To 1.5 g of boric anhydride (Eastman Kodak Co., Cat. #2685 or equivalent) contained in a 42 ml Parr bomb, elec. ignition type, add from a small weighing buret ca 0.25–0.30 g sample contg 0.030–0.034 g Cl. (When a sample larger than 0.30 g is required, 2.5 g boric anhydride should be used. In no case should a sample larger than 0.6 g be taken.) Mix well with a thin stirring rod. Measure 15 g of calorimetric grade Na_2O_2 in a standard measuring dipper, add a small portion to contents of the bomb, and stir. Add balance of Na_2O_2 and thoroly mix by stirring with rod. Withdraw rod and brush free of adhering particles. Quickly cut or break off lower 1½" of stirring rod and imbed in fusion mixture. Prep. head by heating fuse wire momentarily in a flame and immersing it into a small quantity of sucrose. One mg sucrose is sufficient to start the combusion. Assemble bomb and ignite in usual manner.

Place ca 100 ml of distd H_2O in a 600 ml beaker and heat nearly to boiling. After cooling bomb, dismantle and dip cover in the hot H_2O to dissolve any of the fusion which may be adhering to the under side. Wash cover with fine jet of distd H_2O catching washings in the beaker. With tongs, lay fusion cup on side in the same beaker of hot H_2O, covering it immediately with watch glass. After fused material has dissolved, remove cup and rinse with hot H_2O, cool soln, add several drops of phenolphthalein indicator, neutralize with concd HNO_3 and add 5 ml in excess. Det. Cl by electrometric titration or by Volhard procedure **5.148(a)** or **(c).**

Run a blank which includes all reagents used.

(5) The following use-dilution method for evaluating disinfectants was adopted, first action:

METHOD I

(Using *Salmonella cholerasuis*)

REAGENTS

(a) *Culture media.*—**(1)** *Nutrient broth.*—Boil 5 g beef extract (Difco), 5 g NaCl, and 10 g Armour peptone (quality specially prepared for disinfectant testing) in 1 l H_2O 20 min., adjust to *p*H 6.8 and make to vol. with H_2O. Filter thru paper, place 10 ml quantities in 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lb pressure for 20 min.

(2) *Nutrient Agar.*—Dissolve 1.5% Bacto agar (Difco) in nutrient broth and adjust to *p*H 7.2–7.4; place 15 ml quantities in 25×150 mm tubes, plug with cotton, sterilize at 15 lb pressure for 20 min, slant and allow to solidify at room temp.

(3) *Subculture media.*—Use (a), (b), or (c), whichever gives lowest result:

(a) Nutrient broth described in **(a)(1).**

(b) *Fluid thioglycollate medium U.S.P. XIII.*—Mix 0.75 g l-cystine, 0.75 g agar, 2.5 g NaCl, 5.5 g dextrose, 5.0 g H_2O-sol. yeast ext., 15.0 g pancreatic digest of casein with 1 l H_2O; heat to dissolve on H_2O bath, add 0.5 g Na thioglycollate or 0.3 g thio-glycollic acid, and adjust with *N* NaOH to *p*H 7.0 ±0.1; reheat without boiling and filter thru moistened filter paper; add 1.0 ml freshly prepd 0.1% Na

resazurin soln; tube in 10 ml quantities in 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lb steam pressure 20 min.; cool at once to 25° and store at 20–30°.

(c) *Lecithin broth.*—Dissolve 0.7 g lecithin (azolectin) and 5.0 g sorbitan monooleate ("Tween 80") in 400 ml hot H_2O and boil until clear; add 600 ml aq. soln of 5.0 g beef extract (Difco), 10.0 g peptone (Armour), and 5 g NaCl, and boil 10 min.; adjust with *N* NaOH and/or *N* HCl to *p*H 7.0±.2 and filter thru coarse filter paper; tube in 10 ml quantities in 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lb pressure 20 min.

With oxidizing products and products formulated with toxic compounds contg certain heavy metals like Hg, (b) will usually give the lowest result. With products contg cationic surface active materials, (c) will usually give lowest results.

(b) *Test organism, Salmonella cholerasuis* (A.T.C.C. 10708).—Carry stock culture on nutrient agar slants. Transfer once a month and incubate new stock transfer 2 days at 37°, then store at room temp. From stock culture inoculate tube of nutrient broth and incubate at 37°. Make 3 consecutive 24 hr transfers, then inoculate tubes of nutrient broth (2 for each 10 carriers to be tested) using one loop of inoculum with each tube, incubate at 37° for 44–48 hrs.

(c) *Phenol.*—Use phenol, U.S.P., which has congealing point 40° or above. Use 5% soln as stock soln and keep in well stoppered amber bottles in relatively cool place, protected from light. Standardize with 0.1 *N* K or Na bromide-bromate soln, **39.18.**

(d) *Sterile distilled water.*—Prep. stock supply distd H_2O in 1 l flasks, plug with cotton, sterilize at 15 lb pressure for 20 min. and use to prep. dilns of medicants.

(e) *Asparagine.*—Make stock supply of 0.1% soln of asparagine ("Bacto") in distd H_2O in Erlenmeyer flasks of convenient size, plug with cotton, and sterilize at 15 lb for 20 min. Use to cover metal carriers for sterilization and storage.

(f) *N* NaOH.—Maintain stock supply of NaOH soln of ca *N* (4%) for cleaning metal carriers prior to use.

APPARATUS

(a) *Glassware.*—1, 5, and 10 ml volumetric pipets; 1, 5, and 10 ml Mohr pipets graduated to 0.1 ml or less; 100 ml stoppered cylinders graduated in 1 ml divisions; Pyrex lipped test tubes 25×150 mm; straight side Pyrex test tubes 20×150 mm; 15×110 mm petri dishes, 100 ml, 300 ml, and 1 l Erlenmeyer flasks. Plug all tubes and flasks with cotton. Sterilize all glassware 2 hrs in hot air oven at 180° employing closed metal containers for pipets and petri dishes.

(b) *Water bath.*—Insulated relatively deep H_2O bath with cover having at least 10 well spaced holes which admit medicant tubes but not their lips.

(c) *Racks.*—Any convenient style. Conventional wire racks or blocks of wood with deep holes are satisfactory. Have holes well placed to insure quick manipulation of tubes.

(d) *Transfer loops and needles.*—**(1)** Make 4 mm (inside diam.) single loop at end of 2–3 inch Pt alloy wire No. 23 B&S gauge. Have other end in suitable holder (glass or Al rod). Bend loop at a 30° angle.

(2) Make 3 mm right angle bend at end of 2–3 inch nichrome wire No. 18 B&S gauge. Have other end in suitable holder (glass or Al rod).

(e) *Carriers.*—Polished stainless steel cylinders (penicillin cups)* with an outside diam. of 8±0.1 mm.

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* See Federal Register, Vol. 12, No. 67, p. 2217, April 4, 1947.

† May be purchased from Erickson Screw Machine Products Co., 25 Lafayette Street, Brooklyn, New York.

(f) *Petri dishes.*—Have ca 6 sterile petri dishes matted with a layer of S&S No. 597, 9 cm filter papers.

DETERMINATION

Soak ring carriers overnight in *N* NaOH soln, rinse with tap H_2O until rinse H_2O gives neutral reaction to phenolphthalein, then rinse 2 times with distd H_2O; place cleaned ring carriers in multiples of 10 in cotton plugged Erlenmeyer flasks or 25×150 mm cotton plugged Pyrex test tubes, cover with 0.1% soln of asparagine (e), sterilize at 15 lb for 20 min., cool and hold at room temp. Transfer 20 sterile ring carriers using flamed nichrome wire hook into 20 ml of a 44-48 hr nutrient broth test culture in a sterile 25×150 mm medicant tube. After 15 min. contact period, remove cylinders, using flamed nichrome wire hook, and place on end in vertical position in a sterile petri dish matted with filter paper. Place in incubator at 37° and allow to dry for no less than 10 min. and no more than 60 min. Hold the broth culture for detn of its resistance to phenol by the phenol coefficient method.

From the 5% stock soln make 1–90 and 1–100 dilns of the phenol directly into medicant tubes. Place tube for each diln in H_2O bath and allow to come to temp. Make stock soln of the germicide to be tested in sterile glass stoppered cylinder. From this soln make 10 ml dilns to be tested depending upon the phenol coefficient found and/or claimed against *S. typhosa* at 20° directly into each of ten 25×150 mm medicant tubes, and then place the 10 tubes in the H_2O bath at 20° and allow to come to temp. Det. the diln to be tested by multiplying the phenol coefficient number found and/or claimed by 20 to det. the number of parts of H_2O in which one part of germicide is to be incorporated.

Add 0.5 ml of the test culture suspension to the 1–90 diln of the phenol control after a 30 sec. interval, add 0.5 ml to the 1–100 diln of the control, using sterile cotton plugged pipets. After adding culture, agitate tubes gently but thoroly to insure even distribution of bacteria and replace in bath; 5 min. after seeding first medicant tube, transfer 1 loopful of mixt. of culture and dild phenol from medicant tube to corresponding subculture tube. At end of 30 sec. interval, transfer loopful from second medicant tube; 5 min. after making first set of transfers begin second set of transfers for 10 min. period; and finally repeat for 15 min. period. Use technique of loop sampling, flaming loop and mouths of tubes and agitating medicant and subculture tubes as outlined in phenol coefficient method. Incubate subcultures at 37° 48 hrs and read results. Resistance in the 44–48 hr culture of *S. cholerasuis* should fall within range specified for the 24 hr culture of *S. typhosa* in the phenol coefficient method.

Add one contaminated dried cylinder carrier to each of the 10 tubes of the use-diln of the germicide to be tested at 1 min. intervals. Thus, by the time the 10 tubes have been seeded, 9 min. will have elapsed plus a 1 min. interval before transfer of the first carrier in series to an individual tube of subculture broth. This interval is a constant for each tube with the prescribed exposure period of 10 min. The 1 min. interval between transfers allows adequate time for flaming and cooling nichrome wire hook and making transfer in a manner so as to drain all excess medicant from carrier. Flame lips of medicant and subculture tubes in conventional manner. Immediately after placing carrier in the medicant tube swirl tube 3 times before placing it back into bath. Shake subculture tubes thoroly, incubate 48 hrs at 37°, and report results as + (growth) or − (no growth) values. Where there is reason to suspect that lack of growth at the conclusion of incubation period may be due to bacteriostatic action of medicant adsorbed on carrier which has not been neutralized by subculture medium employed, each ring shall be transferred to a new tube of sterile medium and reincubated for an addnl period of 48 hrs at 37°. Results showing no growth on all 10 carriers would confirm the phenol coefficient number found.

Results showing growth on any of the 10 carriers should be considered as indicating the phenol coefficient number to be an unsafe guide to the diln for use. In the latter case, the test should be repeated using lower dilns of the germicide under study. The maximum diln of the germicide which kills the test organism on the 10 carriers in the 10 min. interval would represent the maximum safe use-diln.

METHOD II

(Using *Micrococcus pyogenes* var. *aureus.*)

Proceed as directed in Method I except to change phenol dilns and test organism. Use culture of *M. pyogenes* var. *aureus* F.D.A. 209, A.T.C.C. No. 6538 having at least the resistance specified for the 24 hr culture at 20° in the phenol coefficient method.

6. PLANTS

(1) The following nitrosocresol method[1] for the determination of cobalt in plants was adopted, first action:

REAGENTS

(Make all distns in Pyrex glass stills with ground glass joints. Store reagents in glass-stoppered Pyrex bottles.)

(a) *Redistilled water.*—Distill twice, or pass thru a column of an ion-exchange material (IR-100A or equivalent) to remove heavy metals.

(b) *Hydrofluoric acid.*—Reagent grade, 48%. Procurement in vinyl plastic bottles is advantageous.

(c) *Perchloric acid.*—Reagent grade, 60%. No further purification necessary.

(d) *Hydrochloric acid.*—1+1. Add an equal vol. reagent grade concd HCl to distd H_2O and distill.

(e) *Ammonium hydroxide.*—1+1. Distill concd NH_4OH into an equal vol. of redistd H_2O.

(f) *Ammonium hydroxide.*—0.02 *N*. Add 7 ml NH_4OH (1+1) to 2.5 l redistd H_2O.

(g) *Carbon tetrachloride.*—Distill over CaO, passing the distillate thru an acid-washed filter paper.

(h) *Dithizone.*—Dissolve 0.5 g dithizone in 600–700 ml CCl_4 (tech. grade is satisfactory). Filter into a 5 l separatory funnel contg 2.5–3.0 l 0.02 *N* NH_4OH, shake well, and discard CCl_4 layer. Shake with 50 ml portions redistd CCl_4 until the CCl_4 phase as it separates has a pure green color. Add 1 l redistd CCl_4 and acidify slightly with distd 1+1 HCl. Shake the dithizone into the CCl_4 layer and discard aq. layer. Store in cool, dark place, preferably a refrigerator.

(i) *Ammonium citrate soln.*—40%. Dissolve 800 g citric acid in 600 ml distd H_2O, and while stirring add slowly 900 ml of concd NH_4OH. The reaction is exothermic and care must be taken to prevent splattering. Adjust *p*H to 8.5 if necessary. Dil. to 2 l and ext. with 25 ml portions of dithizone soln until the aq. phase stays orange-colored and the CCl_4 remains predominantly green. Then ext. soln with CCl_4 until all of the orange color is removed.

(j) *Phenolphthalein.*—Dissolve 1 g in 100 ml distd 95% ethanol.

(k) *Hydrochloric acid.*—0.1 *N*. Dil. 16.6 ml 1+1 HCl to 1 l with redistd H_2O.

(l) *Hydrochloric acid.*—0.01 *N*. Dil. 100 ml 0.1 *N* HCl to 1 l with redistd H_2O.

(m) *Sodium hydroxide soln.*—*N*. Dissolve 40 g reagent grade NaOH in 1 l redistd H_2O.

(n) *Sodium borate buffer.*—*p*H 7.8. Dissolve 20 g boric acid in 1 l redistd H_2O.

[1] *This Journal*, 34, 710 (1951).

Add 50 ml *N* NaOH and adjust *p*H if necessary. Equal volumes sodium borate buffer and 0.01 *N* HCl should give a soln of *p*H 7.0.

(o) *Sodium borate buffer.*—*p*H 9.1. To 1 l sodium borate buffer, *p*H 7.8, add 120 ml *N* NaOH and adjust *p*H if necessary.

(p) *Skellysolve B.*—(Skelly Oil Company, Chicago, Ill.) Purify by adding 20–30 g/l of silica gel (Davison Chemical Company, Baltimore Md.), let stand for several days and distill.

(q) *Cupric acetate.*—Dissolve 10 g reagent grade cupric acetate in 1 l redistd H_2O.

(r) *o-Nitrosocresol.*—Dissolve 8.4 g anhyd. $CuCl_2$ and 8.4 g hydroxylamine hydrochloride in 900 ml distd H_2O. Add 8 ml *m*-cresol (Eastman Kodak Co., practical grade) and stir vigorously while 24 ml 30% H_2O_2 is added slowly. Stir with motor-driven stirrer for 2 hrs at room temp. (Standing for longer periods results in excessive decomposition.) Add 25 ml concd HCl and ext. the *o*-nitrosocresol with 4 successive 150 ml portions Skellysolve B in large separatory funnel. Then add addnl 25 ml concd HCl and again ext. with four 150 ml portions Skellysolve B. Wash combined Skellysolve B extracts twice with 50–100 ml portions 0.1 *N* HCl and twice with 50–100 ml portions redistd H_2O. Shake soln of *o*-nitrosocresol with successive 50–100 ml portions 1% cupric acetate until aq. phase is no longer deep blood-red. When light purple color is evident, extn is complete. Discard Skellysolve B phase, acidify the aq. soln of the cupric salt with 25 ml concd HCl, and ext. the reagent with two successive 500 ml portions Skellysolve B; wash by shaking with two 150–200 ml portions 0.1 *N* HCl and several 150–200 ml portions redistd H_2O. Store soln of *o*-nitrosocresol in refrigerator at ca 4°. This reagent is stable for 6 months or more.

(s) *Sodium o-nitrosocresol.*—Ext. 100 ml of *o*-nitrosocresol soln by shaking with 2 successive 50 ml portions sodium borate buffer, *p*H 9.1, in separatory funnel. If this is carried out as 2 extns, the resulting reagent is more concd. It is important that the total vol. of *o*-nitrosocresol soln equals the total vol. of buffer.

(t) *Cobalt standard soln.*—Prep. stock soln contg 100 mmg/ml Co by heating $CoSO_4 \cdot 7H_2O$ in an oven at 250°–300° to constant wt (6–8 hrs). Weigh exactly 0.263 g of the $CoSO_4$ and dissolve in 50 ml redistd H_2O and 1 ml concd H_2SO_4. Make to vol. of 1 l. Transfer 5 ml of the stock soln to a 1 l volumetric flask and dil. to vol. with redistd H_2O. This soln contains 0.5 mmg Co/ml and is used as a working standard.

(u) *Hydroxylamine acetate buffer.*—*p*H 5.1. Dissolve 10 g hydroxylamine hydrochloride and 9.5 g anhyd. sodium acetate in 500 ml redistd H_2O. The resulting soln will have a *p*H of 5.0–5.2.

SPECIAL EQUIPMENT

(a) *Platinum dishes.*—Ca 70 ml capacity for ashing.

(b) *Automatic dispensing burettes.*—100 ml capacity, of a type that can be fitted to an ordinary 5 lb reagent bottle and that can be filled by means of an aspirator bulb are most convenient.

(c) *Wooden separatory funnel rack.*—Twelve 120 ml separatory funnel size is convenient for dithizone extns. The rack is fitted across the top with a removable bar padded with sponge rubber to make it possible to shake all 12 separatory funnels as a unit.

(d) *Racks.*—Consisting of two 2″×2″×25″ wooden bars with holes drilled at close intervals to take 50 ml centrifuge tubes fitted with No. 13 standard taper glass stoppers are convenient. These tubes are made by reaming out the neck of Pyrex heavy walled centrifuge tubes (Rockefeller Institute of Medical Research type) with a standard taper carbon rod and grinding to take a standard taper stopper. The

tubes are placed upright in one section, and the other (fitted with sponge rubber discs ½" in thickness in the bottom of the holes) is placed across their tops. The 2 sections are fastened at the ends with removable rubber connectors made from ordinary tubing of a convenient size. This makes it possible to shake any number of tubes as a unit. These tubes are utilized for the reaction of Co with nitrosocresol, the extn of the complex into Skellysolve B, and washing of the Skellysolve B soln.

(e) *Shaking machine.*—A commercial mechanical shaker similar to Model L-2549 in Catalogue G-3 of the George H. Wahmann Manufacturing Company of Baltimore, Md., is satisfactory when modified to give a longitudinal stroke of 2" at a rate of ca 180 strokes per min. This shaker is used in making dithizone extns and in the extn of the Co complex.

(f) *Colorimeter.*—The colorimeter found most satisfactory for this work is described by Ellis and Brandt.[1] The Coleman Model 11 Spectrophotometer can be used for the Co detn. A wave length of ca 345 mμ, using the null-point method, is most satisfactory. With this instrument, the calibration curve deviates slightly from linearity. However, the region of the curve between 0 and 1 mmg Co approaches a straight line. A pair of matched Pyrex glass absorption cells at least 5 cm in length is required. A satisfactory cell is made by the American Instrument Company, Silver Spring, Md. (Catalogue No. 5–997, Style D, horizontal with neck for cork or rubber stopper is recommended.) The outside diam. is 13 mm and the length 5 cm. The capacity is ca 3 ml.

CLEANING OF GLASSWARE

Clean the 120 ml Pyrex separatory funnels for dithizone extns by initially soaking them for 30 min. in hot concd HNO_3 and rinsing several times with distd H_2O. As an added precaution, shake with several portions of dithizone in CCl_4. After use, clean by rinsing with distd H_2O, drain, and stopper to avoid contamination. It is not necessary to clean every time with the acid. The HNO_3 cleaning should be repeated, however, if the blanks are unusually high.

The 50 ml glass-stoppered Pyrex centrifuge tubes are cleaned by soaking for 30 min. in concd HNO_3 followed by several rinsings in distd H_2O.

Pipets are completely submerged in a cylinder of H_2SO_4-$K_2Cr_2O_7$ cleaning soln overnight and then rinsed several times with distd H_2O before suspending upright in a rack to dry.

All other glassware is washed thoroly in detergent and rinsed well with tap H_2O followed by a dipping in H_2SO_4-$K_2Cr_2O_7$ cleaning soln. The cleaning soln is rinsed off with tap H_2O followed by several distd H_2O rinses.

Pt is cleaned by scrubbing with sea sand followed by boiling in 10% HCl for 30 min. and rinsing with distd H_2O several times.

PREPARATION OF SAMPLE

All plant material is first oven-dried for 48 hrs and prepd for ashing by either of the following methods:

(1) Grind material in a Wiley mill equipped with a stainless steel sieve. Thoroly mix by rolling, and sample by quartering.

(2) Using stainless steel shears cut the material by hand fine enough for convenient subsequent sampling.

ASHING OF SAMPLES

Weigh 6 g dry plant tissue into a clean Pt dish. Cover with Pyrex watch glass, and place in cool muffle; heat slowly to 500°C. for overnight. Remove sample and cool. Wet down ash carefully with fine stream of redistd H_2O. From dispensing buret add slowly 2–5 ml $HClO_4$, dropwise at first to prevent spattering. Add ca 5 ml H_2F_2.

[1] Anal. Chem. 21, 1546–8 (1949).

Evap. on steam bath. Transfer to sand bath and maintain at medium heat until fuming ceases. Cover with Pyrex watch glass and return to the partially cooled muffle and heat gradually to 600° C. Allow to remain at this temp. for one hr. Remove sample and cool. Add 5 ml 1+1 HCl and ca 10 ml redistd H_2O. Replace cover glass and warm on steam bath to effect soln. Usually a clear soln essentially free of insol. material is obtained. Transfer sample to 50 ml volumetric flask, washing dish several times with redistd H_2O, and dil. to vol. Mix thoroly to insure homogeneous soln. Pt dishes can ordinarily be used several times between sand and acid cleanings.

DITHIZONE EXTRACTION

Transfer suitable sized aliquot (2–3 g dry material) to 120 ml separatory funnel (use vaseline as stopcock lubricant). Add 5 ml 40% NH_4 citrate soln. Add one drop phenolphthalein and adjust to *p*H 8.5 with 1+1 NH_4OH. If ppt forms add addnl NH_4 citrate. Add 10 ml dithizone in CCl_4 and shake 5 min. Draw off CCl_4 phase into 100 ml beaker. Repeat as many times as necessary using 5 ml quantities of dithizone soln and shaking for 5 min. each time. The extn is complete when aq. phase remains orange and CCl_4 phase remains predominantly green in color. Then add 10 ml CCl_4, shake 5 min., and combine with CCl_4 ext. The final 10 ml CCl_4 should be pure green. If not, extn was incomplete and must be repeated. Add 2 ml $HClO_4$ to combined CCl_4 exts, cover beaker with Pyrex watch glass, and digest on hot plate until colorless. Remove cover glass and evap. slowly to dryness. If sample is heated for any length of time at a high temp. after coming to dryness, losses of Co may occur. Heat only enough to completely evap. to dryness. If free acid remains it will interfere with the next step where *p*H control is important. Add 5 ml 0.01 *N* HCl to residue. Heat slightly to assure soln and transfer to 50 ml glass-stoppered centrifuge tube or 60 ml separatory funnel with redistd H_2O.

DETERMINATION OF COBALT

Add 5 ml sodium borate buffer, *p*H 7.8, to sample and 2 ml freshly prepd sodium *o*-nitrosocresol soln. Add exactly 5 ml Skellysolve B and shake 10 min. Remove aq. phase by moderate suction thru a finely-drawn glass tube. To the Skellysolve B add 5 ml 1% aq. cupric acetate and shake for 1 min. to remove excess reagent. Again remove and discard aq. phase. Wash the Skellysolve B by shaking 1 min. with 5 ml redistd H_2O, removing the aq. layer as before; finally shake the Skellysolve B with 5 ml hydroxylamine-acetate buffer 1 min. to reduce the Fe. Transfer the Skellysolve B soln of the Co complex to a 5 cm absorption tube and read in photoelectric colorimeter using Corning standard thickness filters No. 5860 and 4308, or light band as close as possible to point of maximum absorption, 360 mμ.

BLANKS AND STANDARDS

With each set of detns an ashing blank, a reagent blank, and an appropriate standard curve are included. Since the Lambert-Beer law holds for the Co-*o*-nitrosocresol complex, 3 points, 0.0 mmg (reagent blank), 0.5 mmg, and 1.0 mmg are sufficient to define the standard curve. The % transmission of the 0.0 mmg point should never drop below 90%. If it drops below, the *o*-nitrosocresol should be repurified by alternately transferring to the aq. phase as the Cu salt and to the Skellysolve B phase as the free compound following acidification of the aq. phase. The ashing blank should have transmission reading not more than 2–3% lower than the reagent blank.

It is also advisable to include a standard sample with each set of samples to detect contamination or unusual losses of Co in the procedure. A commercial buckwheat flour containing 0.05 p.p.m. Co has proven satisfactory for this purpose.

CALCULATIONS

Results are expressed in terms of p.p.m. of Co and are based upon the dry wt of sample. A simple formula to express the relationship of the various factors involved is:

$$\frac{\text{Total vol. of soln in ml}}{\text{Dithizone aliquot in ml}} \times \frac{\text{mmg Co}}{\text{Dry weight of sample in g}} = \text{Parts per million.}$$

The value for mmg of Co is obtained from the standard curve less the ashing blank.

(2) The following nitroso-R-salt method for the determination of cobalt in plants was adopted, first action:

REAGENTS

(Use reagents specified in the nitrosocresol method, item 1, and the following:)

(v) *Nitroso-R-salt.*—0.2%. Dissolve 2 g powd. nitroso-R-salt (Eastman Kodak Company) in redistd H_2O and dil. to 1 l.

(w) *Nitric acid solution.*—(1+1). Dil. concd HNO_3 with an equal vol. distd H_2O and redistill in an all Pyrex app. Store in Pyrex bottles.

(x) *Bromine water.*—A satd soln of Br in redistd H_2O.

(y) *Citric acid.*—0.2 *N*. Use special reagent grade Pb-free citric acid.

PREPARATION AND ASHING OF SAMPLES

Follow the nitrosocresol procedure except that a 10 g sample of dry plant tissue is used for ashing thru the sentence, "Usually a clear soln essentially free of insol. material is obtained."

DITHIZONE EXTRACTION

Transfer the entire soln to 120 ml separatory funnel and proceed as directed in the paragraph "Dithizone Extraction" of the nitrosocresol method thru the sentence, "If free acid remains . . . *p*H control is important." Dissolve in 1 ml 0.2 *N* citric acid, transfer to 25 ml volumetric flask and make to vol. with redistd H_2O.

DETERMINATION OF COBALT

Transfer suitable size aliquot of the citric acid soln (ca 8 g dry material) to 50 ml beaker. Evap. to 1–2 ml. Add 3 ml Na borate buffer and adjust pH to 8.0–8.5 with NaOH (check externally with phenol red). Vol. should not exceed 5 ml. Add 1 ml of nitroso-R-salt soln. *slowly with mixing.* Boil 1–2 min. Add 2 ml 1+1 HNO_3. Boil 1–2 min. Add 0.5–1.0 ml satd Br-H_2O, cover with watch glass and let stand warm for 5 min. Boil 2–3 min. to remove excess Br.

Cool and make up to 10 or 25 ml (depending on length of light path in absorption cell). Transfer to absorption cell and read at 500 mμ within an hr. Standards containing 0.5, 1, 2, 3 and 4 mmg Co should be carried through the same procedure as for the unknown beginning with "Determination of cobalt."

(3) The following sodium diethyldithiocarbamate method for the determination of copper in plants was adopted, first action:

REAGENTS

(Use reagents specified in the nitrosocresol method, item 1, and the following:)

(z) *Sodium diethyldithiocarbamate.*—0.1% soln. Freshly prepd in redistd H_2O.

(aa) *Copper standard soln.*—Dissolve 0.3930 g $CuSO_4 \cdot 5H_2O$ in redistd H_2O, add 5 ml H_2SO_4 and make up to a l and mix. Take 10 ml aliquot, add 5 ml H_2SO_4 and make up to a l and mix. 1 ml contains 1 mmg Cu.

DETERMINATION OF COPPER

Transfer an aliquot (1 g dry material) from the citric acid soln obtained from the section, *Dithizone Extraction, nitrosocresol method, item* 2, to 125 ml separatory funnel. Add 2 ml NH_4 citrate and 1 drop phenolphthalein. Add 5 ml of Na diethyldithiocarbamate soln. Add NH_4OH (1+1) until pink. Add 10 ml CCl_4. Shake 5 min. Draw off the CCl_4, centrifuge for 5 min. Transfer to an absorption cell and read with filters (Corning) 3389 and 5113, or at 430 mμ.

Prep. a standard curve with 1, 5, 10, 15 and 20 mmg Cu treated as in the *Determination of Copper.*

(4) The first action micro method for dextrose, **29.61–29.63** (p. 513–514), was adopted first action as applied to plants.

7. BAKING POWDERS

No additions, deletions, or other changes.

8. BEVERAGES: NON-ALCOHOLIC AND CONCENTRATES

No additions, deletions, or other changes.

9. BEVERAGES: DISTILLED LIQUORS

No additions, deletions, or other changes.

10. BEVERAGES: MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS

No additions, deletions, or other changes.

11. BEVERAGES: WINES

No additions, deletions, or other changes.

12. CACAO BEAN AND ITS PRODUCTS

(1) The following revision of method **12.36–12.37** for the determination of lecithin in cacao products was adopted, first action:

(a) Delete **12.36** including Figure 21.

(b) Revise **12.37** to read as follows:

Weigh 5 g prepd sample, **12.1,** into 200 ml volumetric flask, add ca 150 ml $CHCl_3$-absolute alcohol (1+1), and shake occasionally during day. At end of day dil. to vol. with same solvent, pour into 250 ml centrifuge bottle, stopper, and allow to stand overnight. Next day centrifuge with stopper on until clear (ca 15 min. at 1800 r.p.m.) and pipet 100 ml of clear liquid into 500 ml Kjeldahl flask. Place Kjeldahl flask on steam bath, remove solvent with current of air, and det. P_2O_5 as directed in **20.49** and **20.50.** $P_2O_5 \times 11.37 =$ lecithin.

P_2O_5 may be det. by **6.40,** in which case conc. the 100 ml clear liquid in a 250 ml beaker, wash into small crucible with solvent, evap., and proceed with **6.39.** After digesting on steam bath, the crucible must be heated cautiously on a gauze until dry, and the heating continued until frothing ceases and most of the fat has smoked off before ashing in the furnace. Ashing may be done in the beaker in which the ext. is evapd.

13. CEREAL FOODS

(1) The first action method for water-soluble protein-nitrogen precipitable by 40 per cent alcohol, *This Journal,* **35,** 75 (1952), was adopted as official, and method **13.34** (p. 202) was deleted, final action.

(2) The official method for the determination of acetic and propionic acid in bread, *This Journal*, **34,** 64–68 (1951), was revised as follows, and the revised method was adopted as official:

(a) Under "III. APPARATUS," **(b)** change to read: "*Chromatographic tubes* (ca 15×250 mm or ca 15×450 mm) constricted at lower end to ca 4 mm inside diam."

(b) Under "IV DETERMINATION, *Chromatographic Separation:* (a) *Preparation of partition column,*" add the following paragraph at the end of this section (a):

"Where the amount of propionic acid approaches 20 mg in the column and a definite band is observed below the propionic acid band, the long chromatographic tube (450 mm) and ca 10 g silicic acid should be employed. Then take twice the amounts of H_2O, indicator and NH_4OH used for 5 g of silicic acid."

(3) The official methods for lipoids, **13.35** (p. 202), and for lipoid P_2O_5, **13.36** (p. 202), were deleted.

(4) The following methods for lipoids and lipoid P_2O_5 were adopted, first action:

LIPOIDS

Add 15 ml alcohol, 70% by vol. to 5 g of sample (20 mesh or finer) in 250 ml centrifuge bottle. Give bottle gentle rotatory motion so as to moisten all particles with alcohol, stopper firmly (to keep in place during heating), and set in H_2O bath kept at 75–80°C. (Consider that temp. of bath may drop when bottles are introduced.) Heat 15 min. with frequent mixing by same rotatory motion. Immediately add 27 ml alcohol (95%), stopper bottle, and *shake vigorously* 2 min. Cool, add 45 ml ether and shake vigorously 5 min. (Sample should now be in fine state of division.) Centrifuge at ca 1000 r.p.m. for few min. and decant into 250 ml beaker contg some bits of broken porcelain or glass, and rinse off bottle neck with ether. Re-ext. sample with 3 successive 20 ml portions ether, shaking ca 2 min. each time, centrifuging and decanting into beaker contg first ext. Break up sample each time with glass stirring rod, rinsing with ether on removal. Evap. combined ether-alcohol exts just to dryness on steam bath. Drive off any remaining moisture on sides of beaker by placing in drying oven at 100° for 5 min. Dissolve dry ext. in ca 15 ml $CHCl_3$ and filter soln into previously dried and weighed 100 ml Pt dish thru a Knorr extn type tube (20 mm diam.×11 cm long, stem 10 cm long) on an asbestos mat 3–4 mm thick, covered with ca 10 mm layer of sand. Wash sides of dish and tube with 10 ml and two 5 ml portions $CHCl_3$. Free with glass rod any solid ext. adhering to dish to be sure all lipoids are dissolved. Finally wash tube and tip with 5 ml $CHCl_3$. Evap. $CHCl_3$ on steam bath and dry in oven at 100° to constant wt (ca 90 min.). Weigh. Report ext. as lipoids.

LIPOID P_2O_5

Wash sides of the Pt dish with 10 ml $CHCl_3$ to dissolve lipoids, likewise wash sides of dish with 10 ml 4% alc. KOH soln. Evap. to dryness (cautiously) on steam bath, ash 1 hr at 500°. Cover dish with watch glass, add 15 ml HNO_3 (1+9) to make soln definitely acid, heat on steam bath ca 5 min. and filter into 300 ml Erlenmeyer flask. Wash residue and filter with ca 25 ml hot H_2O. Make soln slightly alk. to litmus paper with NH_4OH from Mohr pipet and then slightly acid with HNO_3 (1+9). Keep vol. less than ca 60 ml. Add 20 ml NH_4NO_3 soln, **20.46(b),** and heat in H_2O bath to 45–50°. Add 20 ml freshly prepd and filtered molybdate soln, **2.11,** and proceed under **20.47,** line 6, "and allow flasks to remain in bath 45–50° . . ." Determine blank and make correction.

14. COFFEE AND TEA

No additions, deletions, or other changes.

15. DAIRY PRODUCTS

(1) The following method for the preparation of samples of creamed cottage cheese was adopted as a procedure:

Place 300–600 g sample at ca 15° in the quart cup of a high speed blendor and blend for the min. time (2–5 min.) required to obtain a homogeneous mixt. The final temp. should not exceed 25°. This may require stopping the blendor frequently after channeling and spooning the cheese back into the blades until the blending action starts.

(2) The following statement was added to the official methods for acidity in milk, **15.4** (p. 227), and acidity in cheese, **15.129** (p. 263):

Results may also be expressed as ml 0.1 *N* NaOH/100 g.

(3) The first action methods for residual phosphatase in milk, Method I, **15.40–15.46** (p. 240–2), and in cream, Method I, **15.67** (p. 248), were adopted as official.

16. EGGS AND EGG PRODUCTS

(1) The first action quantitative method for glycerol, **16.27–16.28** (p. 283), was modified as follows and adopted, first action:

REAGENTS

(a) *Sodium tungstate soln.*—Dissolve 10 g $Na_2WO_4 \cdot 2H_2O$ in H_2O and dil. to 100 ml.

(b) *Potassium periodate soln.*—0.02 *M*. Dissolve 4.6 g KIO_4 in ca 500 ml hot H_2O, dil. to ca 900 ml with H_2O, cool to room temp., and make to 1 l. Test for alky by adding 0.02 *N* H_2SO_4 to 25 ml of the soln contg bromocresol purple indicator, **(c)**. Do not use more than 1 drop of the acid to give yellow acid color.

(c) *Bromocresol purple indicator.*—Dissolve 0.1 g bromocresol purple in 100 ml alcohol.

(d) *Calcium oxide, powd.*—reagent grade.

DETERMINATION

(a) *Eggs with no added sugars.*—Weigh accurately by difference ca 2 g well mixed sample, **16.1(a)** or **(b)**, into 100 ml volumetric flask contg 50–75 ml H_2O. Mix, add 2.0 ml Na_2WO_4 soln. Add slowly with continuous mixing 2.0 ml *N* H_2SO_4. Dil. to mark with H_2O, mix well and filter (18.5 cm folded filter). Transfer aliquot of filtrate contg not more than 40 mg glycerol to 300 ml Erlenmeyer flask, and dil. with H_2O to 20 ml if necessary. Add 2 ml 10% NaOH soln, heat to boiling, and boil 30 sec. Cool slightly, add 3 drops bromocresol purple indicator, neutralize with *N* H_2SO_4 (use buret), and add 1–2 drops in excess. Boil 1 min., cool to room temp., and carefully neutralize with 0.02 *N* NaOH, titrating to light purple shade. Transfer neutral soln quantitatively to 100 ml volumetric flask, restricting total vol. to less than 50 ml. (As aid, mark side of flask to indicate vol. of ca 45 ml.) If necessary, add more 0.02 *N* NaOH to maintain light but definite purple color. Continue as directed in **33.48(b)**, beginning "add 50 ml of the KIO_4 soln." Use **33.49(a)** for the detn.

Excess periodate must be present at end of oxidation period. If periodate test is negative, repeat detn, using smaller aliquots.

(b) *Eggs containing added sugars.*—Prepare sample soln as directed in **(a)** above with ca 2 g sample.

Transfer aliquot of filtrate contg not more than 40 mg glycerol to 400 ml beaker. Adjust to vol. of 20 ml by evapn on steam bath or by addn of H_2O. Add 0.5 g powd. CaO, mix and let stand 30 min. at room temp. with occasional mixing. Add 25 ml alcohol, mix and filter with suction, using Büchner funnel and 7 cm S&S 597 paper or equivalent. Rinse beaker, funnel, and paper with several portions of alcohol. Transfer as much as possible of the residue to the paper but do not attempt to remove film of lime salts adhering to beaker. Transfer filtrate quantitatively to original 400 ml beaker, rinsing flask with several portions of H_2O. Evap. filtrate on steam bath to vol. of ca 10 ml. Filter thru 9 cm S&S 597 paper or equivalent, collecting filtrate in 300 ml Erlenmeyer flask. Rinse beaker, funnel, and paper with small quantities of H_2O, restricting total filtrate vol. to 25 ml or less. Add 1 ml 10% NaOH soln to filtrate and complete the detn as directed in **(a)** above beginning ". . . heat to boiling, and boil 30 sec."

17. ENZYMES

No additions, deletions, or other changes.

18. FISH AND OTHER MARINE PRODUCTS

(1) The procedure for the Preliminary Treatment and Preparation of Sample, **18.1(f)** (p. 295), was modified as follows:

(a) Insert as a new paragraph before the last sentence of the first paragraph, "If drained liquid is more . . . " the following heading: "(1) *Clams or scallops.*—" and add at the end of the section after "NOTE" the following paragraph:

"(2) *Oysters.*—Grind meat including liquid 1–2 min. in Waring blendor."

(2) The first action method for total solids, **18.4** (p. 296), was modified by inserting the phrase "(3 hrs for oysters)" after "4 hrs" and the revised method was adopted, first action.

19. FLAVORING EXTRACTS

(1) The official colorimetric method for vanillin, **19.6** and **19.7** (p. 306), was deleted, final action.

(2) The first action photometric method for vanillin, *This Journal,* **34,** 72 (1951); **35,** 77 (1952), was adopted as official for (a) vanilla, (b) vanilla containing added vanillin and/or coumarin, and (c) imitation vanilla.

(3) The first action photometric method for coumarin, *This Journal,* **34,** 73 (1951); **35,** 77 (1952), was adopted as official for (a) vanilla, (b) vanilla containing added vanillin and/or coumarin, and (c) imitation vanilla.

20. FRUIT AND FRUIT PRODUCTS

(1) The official method for citric acid, *This Journal,* **34,** 74 (1951) as corrected, *This Journal,* **35,** 79 (1952), was revised by deleting the last paragraph under "Isolation of Polybasic Acids," "Dilute 20 ml of the isolated acid soln . . . indicator." and the revised method was adopted as official (suspension of the rules).

(2) The first action bitartrate method for total tartaric acid, **20.35–20.36** (pp. 329–340), was revised as follows, and the revised method was adopted as official:

REAGENTS

(a) *Lead acetate soln.*—Dissolve 75 g normal Pb acetate in H_2O, add 1 ml acetic acid and dil. to 250 ml.

(b) *Potassium hydroxide.*—30%.

APPARATUS

Device for filtering at 0°.—Use app. similar to that described under **16.17(d)**.

PREPARATION OF SAMPLE AND REMOVAL OF PECTIN

Take a quantity of sample prepd as described under **20.2** having titratable acidity approximating 3 ml normal acid with solids content not over 20 g. Designate as "*A*" the ml of *N* alkali required to neutralize sample. Adjust vol. of sample to ca 35 ml by evapn or by addn of H_2O, add 3 ml of *N* H_2SO_4 and heat to 50°. Pour adjusted sample into 250 ml volumetric flask, rinse with 10 ml hot H_2O, and finally with alcohol, cool, make to mark with alcohol, shake and let stand until pptd pectin separates leaving a clear liquid, overnight if necessary. Transfer to centrifuge bottle, add 0.2 g filteraid, shake vigorously, centrifuge and decant thru a retentive paper. Cover funnel with watch glass to prevent evapn. Pipette 200 ml of filtrate into centrifuge bottle.

If original sample is alc., it may contain esters of organic acids, and saponification is necessary. Adjust vol. to 35 ml, add "*A*" +3 ml *N* KOH, heat to ca 60°, and allow to stand overnight. Add "*A*" +6 ml of *N* H_2SO_4, transfer to 250 ml volumetric flask and proceed as directed above.

DETERMINATION

To soln in centrifuge bottle add vol. of Pb acetate soln **(a)** equal to "*A*" +3 ml, or in case saponification was made, "*A*" +6 ml, 0.2 g filteraid, shake vigorously 2 min. and centrifuge. Test supernatant liquid with a few drops of Pb acetate soln and if a ppt is formed, add more of the Pb acetate soln, shake, and again centrifuge. Decant and allow to drain thoroly by inverting the bottle several min. To material in centrifuge bottle add 50 ml 80% alcohol, shake vigorously to disperse the ppt, add 150 ml more 80% alcohol, shake, centrifuge, decant and drain. To Pb salts in centrifuge bottle add ca 150 ml H_2O, shake thoroly, and pass in H_2S to satn. Unsatn is indicated by the presence of a partial vacuum obtained by stoppering bottle, shaking, and observing partial vacuum when carefully removing stopper. Transfer to 250 ml volumetric flask, dil. to mark with H_2O, and filter thru folded paper. Transfer 100 ml of clear filtrate to a 250 ml I flask, tared with 2 or 3 glass beads. (A Harvard trip balance sensitive to 0.1 g is convenient.) Evap. on gauze over flame to ca 30 ml, remove from flame, add a second 100 ml aliquot, and evap. to 19 g (±0.5 g). Neutralize with 30% KOH, one drop at a time, using phenolphthalein indicator and add *one* drop in excess. Add 2 ml acetic acid, 0.2 g of filteraid (Celite 545 is satisfactory), and slowly with agitation 80 ml of 95% alcohol. Cool in cracked ice-salt mixt., shake vigorously 2 min., place in refrigerator and hold overnight at 0°C. Cover the filtering disc (see "Apparatus") with a thin layer of asbestos and place over it a thin layer of filteraid. Place cracked ice in the outer funnel, wash the filter mat with ice-cold alcohol and allow to stand a few min. to cool the filter thoroly. Swirl the flask to suspend the filter aid and ppt, and filter at 0°, sucking the mat dry. (Filtrates and washings should be used for *l*-malic acid detn.) Wash the stopper with ca 15 ml ice-cold 80% alcohol, allowing the wash liquid to run into the

pptn flask. Stopper and shake to wash the flask well. A stirring rod bent at a 45° angle 1" from end helps in washing the inside of the filter tube. Conduct the wash liquid completely around the inside of the filter tube and suck dry. Wash flask and filter tube with 2 portions 15 ml each of ice-cold 80% alcohol. While filtering, keep the flask cold with cracked ice. Remove ice from outer funnel, and transfer ppt and pad to pptn flask with boiling CO_2 free H_2O. Heat almost to boiling, and titrate with 0.1 *N* alkali, using phenophthalein indicator. 1 ml 0.1 *N* alkali = 0.015 g tartaric acid. Tartaric acid ÷ 0.64 = tartaric acid in sample taken.

(3) The following method for *l*-malic acid, applicable in the absence of iso-citric acid, was adopted as a first action alternate method:

LAEVO-MALIC ACID

(Not applicable in presence of iso-citric acid—blackberry)

Conc. filtrate from tartaric acid detn (item 2 above) to ca 5 ml on steam bath. (A jet of air over surface of liquid speeds evapn and prevents danger of loss by bumping.) Cool and add NaOH (1+1) a drop at a time until alk. to phenolphthalein and then add just enough *N* acetic acid to discharge phenolphthalein color. Transfer to a 25 ml volumetric flask and make to mark with H_2O. Pour soln into a fine porosity sintered filter tube containing a mat of carbon several mm thick. (Merck's activated charcoal for decolorizing and Nuchar W have been found satisfactory.) With pressure, force the liquid slowly thru the disc, 1 or 2 ml/min., into a 50 ml flask. If the soln is not colorless, pass thru another fresh mat of carbon. Mix soln and polarize in a 200-mm tube at room temp. using white light. Return soln in polariscope tube to remainder in flask. Add 2.5 g of finely powd. uranium acetate, *protect from light*, and shake in a machine for 0.5 hr. Filter on a retentive paper in the *dark*, mix and polarize as before. Do not allow the treated soln to be exposed to light, which causes the uranium complex to become insoluble, and, if this is filtered off, loss of malic acid will occur. The algebraic difference between the readings in degrees Ventzke, multiplied by the factor 0.0153 gives g *l*-malic acid in the sample taken for the tartaric acid detn.

21. GELATIN, DESSERT PREPARATIONS, AND MIXES

(1) The first action methods for jelly strength, **21.6** (p. 339) and **21.12** (p. 340), were adopted as official.

22. GRAINS AND STOCK FEEDS

(1) The first action method for cobalt in mineral feeds, *This Journal*, **35**, 79 (1952), was adopted as official.

(2) The following method for crude fat in baked dog food was adopted, first action:

Proceed as directed under **13.19** thru: "Evap. ethers slowly on steam bath," then continue as follows: Redissolve fat residue in 20 ml ether. Filter thru a small fat-free filter paper into a 50–100 ml beaker that has been previously dried at 100°, cooled in air, and weighed against a counterpoise similarly treated. Using two 10 ml portions ether, rinse original vessel and transfer to filter paper. Evap. ether slowly on steam bath, then dry the fat in a drying oven at 100° to constant wt (ca 90 min.), cool in air, and weigh against counterpoise as before.

23. MEAT AND MEAT PRODUCTS

(1) The official method for moisture in meat, **23.2** (p. 359), was revised to read as follows:

Proceed as directed under **22.3.** (Not suitable for high fat products such as pork sausage.)

(2) The following air drying methods for moisture in meat were adopted. first action:

(a) Dry, with lids removed, quantity of sample representing ca 2 g of dry material 16–18 hrs at 100–102° in air oven (mechanical convection preferred). Use covered Al dish at least 50 mm in diam. and not exceeding 40 mm in depth. Cool in desiccator and weigh. Report loss in weight as moisture.

(b) Dry, with lids removed, quantity of sample representing ca 2 g of dry material to constant wt (2–4 hrs depending on product) in a mechanical convection oven at ca 125°. Use covered Al dish at least 50 mm in diam. and not exceeding 40 mm in depth. Avoid excessive drying. Cover and cool in desiccator and weigh. Report loss in wt as moisture. (Note: dried sample is not satisfactory for subsequent fat detn.)

24. METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

(1) The first action method for mercury, *This Journal*, **35**, 80 (1952), was adopted as official.

25. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

26. OILS, FATS, AND WAXES

No additions, deletions, or other changes.

27. PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) The following test for purity of bromophenol blue reagent was inserted in the bromophenol blue method for quaternary ammonium compounds, **27.34** (p. 464):

REAGENTS

D.C. 12.—Lauryldimethylbenzylammonium chloride, or other solid quaternary ammonium compound.

Bromophenol blue.—Should pass the following test for purity:

Place 20 mg bromophenol blue in 125 ml separatory funnel, add 50 ml ethylene chloride, 5 ml 1% Na_2CO_3 and shake until dissolved. Let stand until the mixt. separates into 2 layers and observe. The lower layer should be colorless, the upper purple. Now add 10 ml of soln contg 0.1 mg D.C. 12 or other quaternary ammonium compound, again shake and allow mixt. to settle. The lower layer should have a clear blue color. Draw off lower layer and examine in spectrophotometer. The absorption should be greatest at ca 608 mμ. Compare absorption curve with that of a sample purified as directed below. If test gives a yellow or green color or if absorption curve is essentially different from that referred to above, purify as follows:

PURIFICATION

Place 2 g solid bromophenol blue in 400 ml beaker and dissolve in 25 ml of 1% Na_2CO_3. Transfer to a 1-liter separatory funnel using ca 300 ml H_2O. Add 500 ml

ethylene chloride and shake. Add 1 ml of a soln contg 10 mg of D.C. 12 or other quaternary ammonium compound and shake until thoroly extd. If lower layer is yellow, repeat addn of D.C. 12 soln in 1 ml portions with shaking until upon sepn of the 2 layers, the bottom one has a greenish tint. Draw off lower layer and discard. Add to separatory funnel 200 ml ethylene chloride and 1 ml of D.C. 12 soln and shake. This time the lower layer should have a clear blue color. If that layer is green, draw off and repeat addn of ethylene chloride and D.C. 12 until a blue soln is obtained. Wash aq. layer with 100 ml portions ethylene chloride until lower layer is colorless or only faint blue. Acidify aq. layer with HCl and ext. the yellow ppt with ethylene chloride until the aq. soln has only a faint yellow color. Distil off most of the ethylene chloride and permit the remainder to evap. spontaneously in a beaker. Grind the residual powder. Test portion for purity as directed above and if suitable, use as reagent.

(2) The following qualitative test for quaternary ammonium compounds in milk was adopted, first action:

REAGENTS

Use reagents of **27.34** (as revised in item (1) above).

TEST

Pipet 25 ml milk into 250 ml volumetric flask contg 10 mg bromophenol blue, and agitate until the solid reagent is dissolved. Gradually add 50 ml acetone with shaking, then add, dropwise, 1 ml HCl (1+1). This should produce a bright yellow color in the mixt.; if not, add more HCl until a bright yellow color is produced and then add an excess of 0.2–0.3 ml. Gradually, with continuous mixing, dil. to the mark with acetone. Mix, let stand 30 min. and filter thru a folded filter. Measure 200 ml of filtrate in a graduated cylinder and pour into 500 ml separatory funnel, fill the cylinder to the same mark with H_2O and add to the separatory funnel. Wash the acetone-water mixture by shaking with 3 successive 50 ml portions of petr. ether. When sepd, pour each portion of petr. ether thru a filter paper and reserve the paper for filtration of the ethylene chloride ext. later. Evap. the acetone-water soln on steam bath under a current of air until the vol. is reduced to 100 ml or less and the odor of acetone is gone. Cool, transfer to 250 ml separatory funnel with H_2O (reserve the beaker) and add 5 ml of HCl (1+1).

Pipet 50 ml ethylene chloride into the separatory funnel and shake for 1–2 min. Draw off lower layer into the beaker used for the evapn in such a manner as to wash down its sides, and return this liquid to the separatory funnel, washing out the beaker with a little H_2O; again shake 2–3 min., let stand until clear and draw off the lower layer thru the filter paper reserved above into a 125 ml separatory funnel contg 10 ml Na_2CO_3 soln. Stopper, invert the separator and shake carefully for 2–3 min. using a rotary motion. Reverse the funnel to its normal position and let stand to sep. The top layer will be the usual purple color of an alk. soln of strong bromophenol blue: a blue color in the lower layer is a positive test for quaternary. To better observe the color, draw off the lower layer into a glass stoppered flask contg 1–2 g of anhyd. granular (not powd.) Na_2SO_4 which will absorb on contact any drops of the purple soln which may unavoidably find their way into the flask. Decant ethylene chloride layer into another vessel if necessary to avoid any color reflected from the colored salt in the flask. The ethylene chloride layer must not be filtered, since most filter papers contain sufficient residual acid to change the blue color of the bromophenol blue quaternary ammonium salt complex to a practically invisible yellow.

(3) The following qualitative test and spectrophotometric method for the determination of dehydroacetic acid in cheese was adopted, first action:

QUANTITATIVE METHOD

REAGENTS

(a) *Dehydroacetic acid.*—Eastman grade or equivalent.
(b) *Chloroform.*—Analytical grade.
(c) *Hydrochloric acid.*—Ca 1 *N* soln.
(d) *Sodium hydroxide.*—Ca 0.5 *N* soln.

DETERMINATION

Weigh a 50–60 g sample of cheese, to the nearest 0.1 g, place in Waring blendor cup, and comminute (covered) with 80 ml $CHCl_3$ for 3 min., scraping down the walls and cover once during the operation. Place a filter paper on a 2–3″ diam. sintered glass Büchner funnel (if sintered glass funnel is not available, use ordinary Büchner funnel), transfer the cheese to the funnel with a spatula, and filter the $CHCl_3$ with suction. Return cheese cake and filter paper to cup, add 80 ml $CHCl_3$, blend for 1 min. and filter again into the same flask. Use a fresh filter paper for each filtration. Repeat the extn and filtration for the third time with 80 ml portion of $CHCl_3$. Wash sides of filter and cake once with 25 ml $CHCl_3$. A greater portion of the $CHCl_3$ may be removed if the cheese cake is compressed.

Transfer combined $CHCl_3$ filtrates to 500 ml separatory funnel. Rinse filtering flask with 2 small portions of $CHCl_3$ and add to funnel. Ext. $CHCl_3$ soln with ca 33 ml 0.5 *N* NaOH. Transfer the $CHCl_3$ layer to 600 ml beaker and the caustic layer to 300 ml Erlenmeyer flask. Return the $CHCl_3$ to the separatory funnel and repeat the above caustic extn twice. An emulsion may be formed during extn, but most of it will break on standing. Transfer this emulsified layer to the alkali soln only in the final extn. Acidify the caustic ext. with 70 ml 1 *N* HCl, and rapidly aerate for such time as required to remove dissolved $CHCl_3$ (5–10 min.). To check complete removal of $CHCl_3$, smell the top of the flask while aerating. Be sure to remove all the $CHCl_3$ by aeration, or low values will be obtained. Filter the soln thru a medium or fine porosity sintered glass funnel fitted with filter paper and make to vol. with H_2O in 500 ml volumetric flask. If this soln is turbid, clarify by re-filtering thru a fine filter or an asbestos pad.

Prepare a reagent blank by extg 250 ml $CHCl_3$ with the alkali, adding the acid to the ext., aerating, and making to vol. with H_2O.

Place a portion of the reagent blank in one cuvette and a portion of the sample in the other. Determine the optical density of the soln at 307 mμ with a Beckman DU spectrophotometer. Dil. the sample soln if necessary to obtain a reading in the range covered by the standard curve. (The ordinary range of diln for absorption readings is from no diln to a diln of 1–5.)

STANDARD CURVE

For prepn of the standard curve use a fresh dehydroacetic acid soln, as low readings are obtained from older solutions. Weigh exactly 100 mg dehydroacetic acid and transfer to 100 ml volumetric flask. Dissolve the acid in ca 50 ml of H_2O +4 ml 0.5 *N* NaOH. Dil. to vol. with H_2O and mix. Pipet 1.0, 3.0, and 5.0 ml (1.0, 3.0, and 5.0 mg dehydroacetic acid) aliquots of this stock soln into separate 500 ml volumetric flasks. To each add the equivalent of ca 100 ml 0.5 *N* NaOH and 70 ml *N* HCl, dil. to vol., and mix. Det. optical density of the solns at 307 mμ. using a reagent blank prepd as above. (With reagent blank in position, the instrument switch

on "1," and optical density at 0, adjust slit and sensitivity to meter balance and proceed with the other readings.) Plot optical density vs. mg dehydroacetic acid per 500 ml prepd soln.

Calc. dehydroacetic acid to parts per million of cheese:

$$\text{p.p.m.} = \text{mg per 500 ml} \times \frac{1000}{\text{wt sample}}$$

QUALITATIVE TEST

REAGENT

(a) *Salicylaldehyde solution.*—10 ml salicylaldehyde dissolved in 95% alcohol and dil. to 50 ml.

TEST

Transfer the dehydroacetic acid soln remaining in the 500 ml flask after the spectrophotometric quantitative detn to a 1 liter separatory funnel. Add 100–125 ml ether, A.C.S., and shake vigorously. Allow to sep., draw off the aq. layer, and discard. Draw off the ether into 125 ml Erlenmeyer flask, taking care not to include any emulsion or H_2O. Evap. ether ext. to dryness on steam bath and dissolve residue in 1 ml 0.5 *N* NaOH. Pour the 1 ml of alk. soln into a test tube (do not rinse flask) add 0.5 ml alc. salicylaldehyde soln, and 1 ml NaOH (1+1). Mix and place in boiling H_2O bath for 5 min. Remove tube from bath, add 2 ml H_2O, and observe color. Include a reagent blank and a control contg 0.2 or 0.3 mg dehydroacetic acid for comparison. With as little as 10 p.p.m. or less of dehydroacetic acid in cheese, a red or orange color is obtained. The intensity of the color is approximately proportional to the quantity of dehydroacetic acid present.

28. SPICES AND OTHER CONDIMENTS

No additions, deletions, or other changes.

29. SUGARS AND SUGAR PRODUCTS

(1) The table of refractive indices of raffinose hydrate solutions at 20 ° C published in *This Journal*, **35**, 89 (1952) was adopted as official.

30. VEGETABLE PRODUCTS (PROCESSED)

No additions, deletions, or other changes.

31. WATERS, MINERAL AND SALT

No additions, deletions, or other changes.

32. DRUGS

(1) The first action method for sulfaquinoxaline in poultry feeds, **32.191–32.193** (p. 619), was adopted as official.

(2) The following method for the determination of propylene glycol in medicinal mixtures was adopted, first action:

REAGENTS

(a) *Cyclohexane (practical).*—B. p. 81°C.

(b) *Potassium periodate.*—0.02 *M*. Dissolve 4.6 g KIO_4 in ca 500 ml hot H_2O. Dil. to ca 900 ml with H_2O, cool to room temp. and make to 1000 ml. Filter thru sintered glass and store in amber glass bottles in a cool place.

(c) *Potassium arsenite.*—0.02 *N*. Dissolve 4.9455 g reagent As_2O_3, pulverized and dried to constant wt at 100°, in 75 ml *N* KOH. Add 40 g $KHCO_3$, dissolved in

ca 200 ml H_2O, and dil. with H_2O to exactly 1000 ml at 25°. Dilute 200 ml of this soln to 1000 ml with H_2O.

(d) *Starch indicator.*—Mix 0.5 g sol. starch with 10 ml cold H_2O and add 90 ml boiling H_2O. Heat to boiling for ca 5 min. Cool before use.

(e) *Bromocresol purple indicator soln.*—Dissolve 50 mg of bromocresol purple in 100 ml alcohol and filter if necessary.

APPARATUS

(a) *Condenser.*—West type with drip tip and ⅌ inner joint.

(b) *Distilling flask.*—250 ml Erlenmeyer, narrow mouth ⅌ joint.

(c) *Distilling receiver.*—Barrett type, 20 ml capacity, with ⅌ stopcock and ⅌ joints.

DETERMINATION

Separation.—Take aliquot of sample soln contg equivalent of ca 1 g of propylene glycol and transfer to 250 ml Erlenmeyer flask. Add sufficient H_2O to make the total vol. 10–15 ml. Add 75 ml cyclohexane and some glass beads or carborundum chips. Connect flask to distg receiver and to the condenser in an upright position. Fill receiver with cyclohexane, heat flask using a variable heat hot plate and reflux at such a rate that a rapid stream of distillate flows from condenser tip. Reflux ca 8 hrs and cool. Open stopcock and transfer the aq. glycol layer to a 200 ml volumetric flask. Wash down the condenser with several successive portions of H_2O (ca 15 ml each time) collecting the wash H_2O in the receiver and draining it off into the volumetric flask. Dil. to vol. with H_2O and mix well.

Periodate oxidation.—Transfer an aliquot of the aq. soln contg not more than 45 mg propylene glycol to 100 ml volumetric flask. (The sample soln should be neutral to bromocresol purple indicator soln.) Add 50 ml 0.02 *M* KIO_4 soln. Dil. to 100 ml with H_2O and allow to stand one hr.

Titration.—Transfer 50 ml aliquot of the oxidized mixture to an Erlenmeyer flask. Add 1 g $NaHCO_3$ and 0.5 g KI, swirl and titrate with 0.02 *N* $KAsO_2$ soln until the color of the soln fades to light yellow. Add 3 ml starch soln and titrate to the disappearance of the blue color. Standardize 25 ml of the 0.02 *M* KIO_4 by the same titration procedure (Blank). The difference between the two titrations is a measure of the amount of periodate reduced. 1 ml 0.02 *N* $KAsO_2 = 0.76$ mg propylene glycol.

Correction for glycerol if present.—Transfer 25 ml aliquot of the oxidized mixture to an Erlenmeyer flask and add 2 drops bromocresol purple indicator soln. Titrate with 0.02 *N* NaOH to a light purple end point. Apply an appropriate correction for any acidity in the 0.02 *M* KIO_4 soln, titrating 25 ml of 0.02 *M* KIO_4 by the same procedure (Blank). 1 ml 0.02 *N* NaOH = 1.84 mg glycerol

Calculations.—A = 2(ml $KAsO_2$ for blank—ml $KAsO_2$ for 50 ml oxidation aliquot) B = 4(ml NaOH for 25 ml oxidation aliquot—2(ml NaOH for blank correction.) Propylene glycol in sample aliquot = (A − 4B) × .00076 g.

(3) The following method for rutin was adopted, first action:

REAGENTS

(a) *Acetic acid.*—Glacial, A.C.S.

(b) *Ethanol.*—U.S.P., 95%.

(c) *Acid-alcohol reagent.*—550 ml ethanol plus 50 ml glacial acetic acid dild to 1 liter with distd H_2O.

(d) *Standard rutin solution.*—(0.02 mg/ml). Dissolve 100 mg (accurately weighed) reference standard rutin* in 50 ml acid-alcohol reagent and transfer to

* Inquiry with regard to reference standard rutin and quercetin should be made of The National Formulary, American Pharmaceutical Association, 2215 Constitution Ave., N.W., Washington 7, D. C.

250 ml volumetric flask with the aid of small amounts of acid-alcohol reagent. Make to vol. with reagent and mix well. Pipet 5 ml aliquot to a second 100 ml volumetric flask and make to vol. with distd H_2O.

(e) *Standard quercetin solution.*—(0.01 mg/ml). Prepare as in (d) using 50 mg quercetin reference standard.*

APPARATUS

(a) *Spectrophotometer.*—Capable of isolating the following wavelengths: 338.5 mμ, 352.5 mμ, and 366.5 mμ with an isolated spectrum not wider than 5 mμ.

(b) *Absorption cells.*—Matched 1 cm.

(c) *Glass stirring rods.*—Of small enough diam. to dislodge material from the tips of 50 ml conical centrifuge tubes.

DETERMINATION

Extraction.—Weigh directly into a 50 ml centrifuge tube the number of tablets required to give 0.05–0.50 g of rutin (not less than 5 tablets). Record the number and wt. (If tablets are coated, after weighing dissolve coating with distd H_2O, discard the aq. washings, and transfer the rutin-containing core to centrifuge tube.) Add 20 ml acid-alcohol reagent and, by means of the stirring rod, break up tablets. After tablets are thoroly disintegrated, heat mixt. in H_2O bath maintained at 70–80°C for 10 min. resuspending the material occasionally by stirring. At end of this period remove stirring rod, rinse with acid-alcohol reagent, and centrifuge mixture at ca 2,000 r.p.m. for 15 min. After centrifugation decant supernatant into 250 ml volumetric flask. Use funnel, decant with one smooth motion and allow the tube to drain for ca 10 sec. While still inverted, rinse mouth of tube with acid-alcohol reagent. Ext. twice more, starting with "Add 20 ml acid-alcohol reagent. . . ." After the third extn dil. combined supernatants to 250 ml with acid-alcohol reagent. Any insol. material may be removed by filtration after diln if the first portions of filtrate are discarded. Depending on the original wt of rutin taken, make a diln with distd H_2O to give a final concn of 0.01–0.03 g/l of rutin. Precipitates forming during this aq. diln may be removed by filtration if the first portions of filtrate are discarded. Discarding the first 15–20 ml of filtrate guards against concn changes due to adsorption.

ABSORPTIMETRY

Det. the absorbancy of the sample soln (A_S) against a blank of distd H_2O at 338.5, 352.5 and 366.5 mμ. Also det. the absorbance of the rutin standard soln (A_R) and the quercetin standard soln (A_Q)† against a blank of distd H_2O at 352.5 and 366.5 mμ. Calc. the following:

$$R_1 = \frac{A_{s,\ 338.5\ m\mu}}{A_{s,\ 352.5\ m\mu}} \quad \text{and} \quad R_2 = \frac{A_{s,\ 366.5\ m\mu}}{A_{s,\ 352.5\ m\mu}}.$$

If $R_1 = 0.914 \pm .009$ and $R_2 = 0.842 \pm .013$, the extd material can be considered pure rutin and the wt of rutin per tablet can be calcd by means of the following equation:

$$\text{mg rutin per tablet} = \frac{A_{s,\ 352.5\ m\mu} \times d \times W \times .02}{A_{R,\ 352.5\ m\mu} \times w}$$

where d = sample diln factor; W = av. wt per tablet; w = wt of sample.

(A value of R_1 beyond its upper limit while R_2 remains within its range indicates an interfering absorption which diminishes rapidly enough to be ineffective at 352.5 mμ. Under this condition the absorbance observed at 352.5 mμ is accepted as

† In the absence of standard quercetin, the values $A_{Q,352.5} = 0.553$ and $A_{Q,366.5} = 0.631$ may be used. Any error introduced by the use of these predetermined values should be of second order.

correct and the rutin content calcd as for pure rutin. An increase in R_2 while R_1 remains within or below its limits usually indicates the presence of quercetin. A simultaneous increase or decrease of both ratios beyond their respective limits indicates an invalidating condition.) The amounts of rutin and quercetin may be calcd by soln of the following simultaneous equations:

$$A_{s,\ 352.5\ m\mu} = \frac{A_{R,\ 352.5\ m\mu} \times r}{.02} + \frac{A_{Q,\ 352.5\ m\mu} \times q}{.01}$$

$$A_{s,\ 366.5\ m\mu} = \frac{A_{R,\ 366.5\ m\mu} \times r}{.02} + \frac{A_{Q,\ 366.5\ m\mu} \times q}{.01}$$

where r = mg rutin per ml in sample soln; q = mg quercetin per ml in sample soln.

33. COSMETICS

No additions, deletions, or other changes.

34. COLORING MATTERS

No additions, deletions, or other changes.

35. EXTRANEOUS MATERIALS: ISOLATION

(1) The following procedure for the preparation of 60% isopropyl alcohol saturated with gasoline was added to the section on special technics, **35.4** (p. 704):

Add ca 150 ml gasoline to each liter of tech. isopropyl alcohol. Mix and dil. with H_2O to 60%. Allow to stand until comparatively clear. Siphon the alcohol from beneath the gasoline layer and filter.

(2) The following changes in the first action method for sediment test of milk, **35.9** (p. 705) as revised, *This Journal*, **35**, 99 (1952), were adopted, first action:

(a) The heading of **35.9(c)**, *This Journal*, **35**, 99 (1952), was changed to: "*Preparation of coarse standard sediment disks.*"

(b) The following method was inserted as **35.9(d)**:

Preparation of Fine Standard Sediment Disks.—Prep. the following mixt., using oven-dried (100°) materials and U. S. Standard sieves.

	Per cent
Cow manure	66.0
Garden soil	28.0
Charcoal	6.0

(All materials thru No. 140 and retained on No. 200 sieve.)

Proceed as directed in **(c)**, beginning, "Place 2.00 g of above mixture . . . "except to use H_2O instead of 50% sucrose for diluting the 10 ml aliquot to 1 liter.

Where **(c)** states "While stirring, pipet definite volumes . . . ," proceed as follows: Det. approx. capacity of funnel on filtering apparatus by pouring H_2O into assembled app. with filter flask air outlet closed. Include H_2O that filters thru as part of capacity of funnel. While stirring, pipet aliquots of sediment suspension into beakers. Add H_2O to make total vol. 20–50 ml less than capacity of funnel, using a max. total vol. of 400 ml and a min. of 60 ml. With filter flask air outlet closed to prevent filtration, mix dild aliquot and pour into funnel fitted with a wet standard disk that passes tests given below. Add 20–50 ml H_2O to beaker and rinse by swirling.

Pour into funnel, keeping lip of beaker touching surface of H_2O if possible. (Rinse H_2O should nearly fill funnel if capacity is 450 ml or less.) Open flask air outlet. After H_2O has filtered thru pad, apply vacuum and aspirate disk for ca 1 min. Remove pad and let dry in covered dish. If sediment is not evenly distributed, pad must be discarded. After some practice with method, ca 75% of pads prepd should be acceptable. No preservative is required. Pads may be coated with dild plastic cement as in **(c)**.

Test sediment disks as follows: Filter 12 mg standard sediment mixture (60 ml aliquot) thru pad as directed above, using clean flask to catch filtrate. Transfer filtrate to beaker. Rinse flask 3 times with H_2O and add rinsings to beaker. Filter filtrate thru 7 or 9 cm S&S No. 589 White Ribbon Paper (or equivalent) that has been washed with ca 200 ml H_2O, dried to constant wt at 100°C. and cooled in covered dish in desiccator before weighing. Rinse beaker and paper thoroly with H_2O. Dry paper to constant wt as directed above. Testing at least three disks, the average wt of sediment passing thru per disk should not exceed 2.8 mg. In addition, standard disk should not appear to have sediment buried beneath surface. Fine standard sediment disks may be prepd and used in any range between 0 and 14 mg.

(c) The present section **35.9(d)** was changed to **35.9(e).**

(3) The first action method for water-insoluble inorganic residue (WIIR) and excreta in peanut butter, **35.25** (p. 712) was deleted and replaced by the following method which was adopted, first action:

Weigh 100 g portion of sample into 250 ml beaker (hooked-lip type), add ca 10 ml petr. ether, and mix thoroly. Continue to add petr. ether, and mix thoroly, until ca 150 ml has been added. Cover, allow mixt. to settle 25 min., and decant 100 ml of the petr. ether layer and floating light tissue, taking care not to lose any coarse peanut tissue. Add ca 125 ml petr. ether to residue and mix. Allow to settle 15 min. and decant 100 ml as before. Repeat with a third ca 125 ml addn of petr. ether, stir, wash down sides of beaker with stream of petr. ether, allow to settle 10 min., and decant 100 ml. Discard all decanted 100 ml portions of petr. ether. Evap. remainder of petr. ether from residue in beaker; gentle heat may be used. Add 150 ml $CHCl_3$ to residue and mix thoroly; cover beaker and allow to settle 20 min. Stir top layer several times during this period. Decant $CHCl_3$ and floating peanut tissue onto 15 cm filter paper in Büchner funnel, being careful not to disturb heavy residue in bottom of beaker. Save all decanted peanut tissue for later detn of light filth. Repeat extn with small quantities of $CHCl_3$, rinsing all particles from sides of beaker. At this point watch for fragments of rodent excreta pellets on top of NaCl in bottom of beaker; do not decant them. (If sample contains considerable peanut skin, it may be necessary to use a mixt. of $CHCl_3$ and just sufficient CCl_4 to float skin particles away from the heavy residue of NaCl, sand, etc.). Dry residue in air.

Add 50 ml HCl (1+35) to residue in beaker, then add 90 ml boiling H_2O and allow to stand 30 min. with occasional stirring, which should dissolve any phosphate, carbonate, or anhydrite ($CaSO_4$) included with the NaCl. Decant liquid thru ashless filter in 60° glass funnel and finally transfer residue with hot H_2O. Test filtrate for SO_4 by adding 5 ml of satd $BaCl_2$ soln. Wash residue on filter several times with hot H_2O. If above test for SO_4 in filtrate was positive, test residue on filter by placing clean beaker or test tube under funnel and treating residue with 25 ml HCl (1+35), adding a little at a time. Test filtrate with 20 drops of satd $BaCl_2$ soln (fine white ppt of $BaSO_4$ indicates presence of anhydrite in residue on filter; allow 5 min. for ppt to appear). Wash residue on filter with hot H_2O until all HCl is removed.

Remove filter paper. Using magnification of 20–40 diam., examine residue micro-

scopically for fragments of rodent excreta pellets (identified by presence of rodent hair fragments in mass), insect excreta pellets, and other filth. Ignite filter paper in tared curcible over medium Bunsen flame or in muffle furnace at ca 500°. Weigh crucible and contents to nearest 0.5 mg. If "WIIR" is excessive and above test indicates that all $CaSO_4$ has not been removed, make quant. detn of either the Ca or the SO_4 in "WIIR" in crucible, as directed in **31.61** and **31.60.** Calc. this wt to $CaSO_4$ and correct wt of "WIIR."

(4) The first action method for light filth in peanut butter, **35.26** (p. 713), was deleted and replaced by the following method which was adopted first action:

Invert over a smooth sheet of paper the 15 cm filter paper from Büchner funnel contg all decanted peanut tissue (item 3, above). Retain 15 cm filter paper to be rinsed later. Break up any caked or lumpy peanut material and dry overnight at room temp. or in an oven at ca 80° for 1 hr. Transfer the dry residue to a 600 ml beaker. Rinse 15 cm filter paper with H_2O, adding washings to beaker. Add 300–400 ml H_2O and stir until smooth. Add the filtered aq. ext. from 5 g of pancreatin and mix. Adjust to *p*H 8 with Na_3PO_4 soln. Readjust *p*H after ca 15 min. and again after ca 45 min. Add 5 drops of formaldehyde (U.S.P.) and digest overnight at 37–40. Cool. Transfer the digested material to a 2 liter Wildman trap flask and add H_2O to make 800–900 ml vol. Trap off twice with 35 and 25 ml gasoline, resp., **35.4(a).** Filter and examine microscopically at ca 30×.

(5) The first action methods for heavy filth and sand in ground cinnamon, **35.83** (p. 730), light filth in ground cinnamon and turmeric, **35.84** (p. 730), light and heavy filth in ground onion powder, **35.85** (p. 730), and light and heavy filth in ground black and white pepper, **35.86** (p. 730) were deleted and replaced by the following methods which were adopted, first action:

35.83 *Ground cinnamon.*

(a) *Heavy filth and sand.*—Weigh 2 g of sample into 50 ml Centrifuge tube and add ca 45 ml CCl_4. Centrifuge 5 min. at 800 r.p.m. in International Size I Type SB Centrifuge, using No. 240 head with arm length of 5.25″ or its equivalent. Stir layer at top of liquid and repeat centrifuging. Decant ca ⅔ of liquid and floating layer and add fresh CCl_4 up to 45 ml. Mix thoroly and again centrifuge. Decant as much of liquid and floating layer as possible without disturbing residue in centrifuge tube. Wash residue onto 11 cm ashless filter with CCl_4. Examine under low-power microscope for insect excreta, rodent excreta, and other filth. Ignite filter and residue in tared crucible and det. sand and soil.

(b) *Light filth.*—Weigh 50 g of sample into 600 ml beaker. Add 300–400 ml of H_2O. Stir until smooth. Add the filtered aq. ext. from 5 g pancreatin and mix. Adjust to *p*H 8 with Na_3PO_4 soln. Readjust *p*H after ca 15 min. and again after ca 45 min. Add 5 drops formaldehyde and digest overnight at 37–40°C. Cool. Transfer digested material to a 2-liter Wildman trap flask and add H_2O to make 800 ml vol. Trap off twice with 25 and 15 ml gasoline, resp., in the usual manner. Combine trappings in beaker. Transfer contents of beaker to trap flask and fill with H_2O. Stir and after 30 min. trap off into beaker and filter. Examine microscopically.

35.84 *Turmeric.*

Light filth.—Weigh 25 g sample into 400 ml beaker. Add 300 ml $CHCl_3$-CCl_4

mixture (1+1), stir thoroly, and allow to stand 15 min. with occasional stirring. Transfer mixture onto 15 cm filter paper in Büchner funnel and rinse with solvent. Dry overnight or in an oven at 80°C. for 1 hr.

Transfer the dry residue to a 600 ml beaker and proceed as directed under **35.83(b).**

35.85 *Onion and Garlic Powder.*

Light and heavy filth.—Weigh 50 g sample into 250 ml, hooked-lip beaker. Add 200 ml CCl_4, stir thoroly, and allow to stand 30 min. with occasional stirring. Decant plant tissue onto 15 cm filter paper in Büchner funnel. Add 100 ml of CCl_4 and repeat decanting until practically no plant tissue remains with sand and soil on bottom of beaker. Transfer residue in beaker to ashless filter paper with stream of CCl_4 from wash bottle and examine for filth. If there is appreciable quantity of residue, place filter paper in tared crucible, ignite, and determine sand and soil.

Dry the residue of plant tissue from the Büchner funnel overnight or in an oven at 80°C. for 1 hr. Transfer to a 2-liter Wildman trap flask. Add 250 ml Tween 80*—60% alc. soln. (40 ml Tween 80+210 ml 60% alcohol). Mix well and let stand 15–30 min. Add 60% alcohol to make 800 ml vol. Trap off twice in 60% alcohol with 75 and 35 ml gasoline, resp., in the usual manner. Allow to stand 1 to 1½hrs for each of the two extractions, however, and avoid stirring except for a few circular upward strokes immediately after having filled the flask with 60% alcohol prior to the standing period. Filter and examine.

35.86 *Ground Black and White Pepper.*

Light and heavy filth.—Weigh 50 g sample into 600 ml beaker. Add 400 ml CCl_4 and allow beaker to stand at least 1 hr, with occasional stirring. Decant pepper and solvent onto 15 cm filter paper in Büchner funnel, leaving any heavy residue of sand and soil in beaker. Repeat decanting with CCl_4 if necessary to secure practically complete sepn of spice materials from any heavy residue. Transfer residue from beaker to ashless filter and examine for filth. If there is appreciable quantity of residue, place filter paper in tared crucible, ignite and det. sand and soil. Wash spice material in the Büchner funnel with $CHCl_3$ and dry overnight or in an oven at 80°C.

Transfer the dry residue to a 600 ml beaker and proceed as directed under **35.83(b).**

35.87 *Ground Capsicums (red and cayenne pepper, chili powder, paprika, etc.).*

Light and heavy filth. Isolate large elements of filth, such as large larvae, adult insects, clumps of webbing, and insect and rodent excreta pellets by sifting pepper thru No. 10 sieve. Filth is retained on sieve.

Weigh 50 g of pepper into 600 ml beaker and add 400 ml petr. ether. Boil gently 30 min. on elec. hot plate, adding petr. ether occasionally to keep vol. constant. Decant petr. ether onto smooth 15 cm filter papers in Büchner funnel. Add 400 ml CCl_4 and allow to stand 30 min. with occasional stirring. Decant pepper and solvent onto same 15 cm filter paper in Büchner funnel, leaving any heavy residue of sand and soil in beaker. Repeat decanting with CCl_4 if necessary to secure practically complete separation of spice materials from any heavy residue. Proceed as directed in **35.86** above, beginning "Transfer residue from beaker to ashless filter . . . etc."

36. MICROBIOLOGICAL METHODS

(1) The following changes in the first action methods for the examina-

* Polyoxyethylene sorbitan monooleate; Atlas Powder Company, Wilmington, Delaware.

tion of eggs and egg products, **36.1–36.11** (p. 736–739) were adopted, first action:

(a) **36.3(a)** was revised to read as follows:

Liquid eggs.—Thoroly mix sample with sterile spoon or sterile elec. stirrer before analysis. Prep. 1–10 diln by aseptically weighing 11 g of egg material into sterile glass or rubber-stoppered bottle, and add 99 g of sterile buffered distd H_2O, **36.11(b)**, or sterile physiological saline, **36.11(c)**, and 1 tablespoon sterile glass shot. Agitate 1–10 diln thoroly to insure complete soln or distribution of egg material in diluent, by shaking each container rapidly 25 times, each shake being an up-and-down movement of ca 1 ft, time interval not exceeding 7 sec. Allow bubbles to escape and transfer representative portion from 1–10 diln for higher serial dilns as needed. Proceed as directed under **36.4–36.9(a)**. Pour all plates and inoculate other media within 15 min. after prepn of first diln in order to prevent growth or death of microörganisms.

(b) **36.3(b)**, *line 3:* replace "(37°)" with "(not over 45° for not over 15 min.)."

(c) **36.3(c)**, *line 2:* delete "in physiological saline soln."

(d) **36.4**, *line 1:* delete "(1–10 to 1–1,000,000 as needed)." *Line 2:* Replace "nutrient agar" with "tryptone glucose yeast agar or milk protein hydrolysate glucose agar."

(e) **35.5(a)**, *lines 3 and 4:* change "37°" to "35°."

(f) **36.5(b)**, *line 2:* change "37°" to "35°." *Under Note:* change "9th ed., 1946" to "latest edition."

(g) **36.6**, "Incidence of Hemolytic Staphylococci and Streptococci" *line 2:* change "6%" to "5%." *Line 3:* change "0.6 ml" to "0.5 ml."

(h) **36.7**, *line 1:* change "**36.11(d)**" to "**36.11(e)**." *Line 3:* change "from 1–10 to 1–100,000" to "as needed." *Line 4:* change "37°" to "35°."

(i) **36.9**, *line 2:* delete "slowly" in the sentence beginning "Add slowly 30.0 ml. . . ."

(j) **36.9(a)**, *line 4:* insert "min. of" between "Stain" and "45 sec." *Line 7:* change "8th Ed., 1941," to "latest Ed."

(k) **36.10**, designate present paragraph as **(c)** and delete "Nutrient Agar," line 2. Add the following sections:

(a) *Tryptone glucose yeast ext. agar, Bacto dehydrated.*—Or the same prepared from ingredients as follows: tryptone 5 g, dextrose 1 g, yeast ext. 2.5 g, agar 15 g, distd H_2O 1000 ml; adjust to *p*H 7.0.

(b) *Milk protein hydrolysate glucose agar, BBL dehydrated.*—Or the same prepared from ingredients as follows: milk protein hydrolysate 9.0 g, dextrose 1 g, agar 15 g, distd H_2O 1000 ml; adjust to *p*H 7.0.

(l) **36.11**: Insert the following section as **36.11(b)**:

(b) *Buffered distilled water.*—To prep. stock soln, dissolve 34 g KH_2PO_4 in 500 ml distd H_2O, add ca 175 ml *N* NaOH and dil. to 1000 ml with distd H_2O. Adjust to *p*H 7.2. Dil. 1 ml stock soln to 800 ml with boiled and cooled distd H_2O.

Change section **(b)** to **(c)**, **(c)** to **(d)**, and **(d)** to **(e)**.

37. MICROCHEMICAL METHODS

(1) The following Carius method for bromine and chlorine was adopted, first action:

CARIUS COMBUSTION METHOD*

REAGENTS

(a) *Fuming nitric acid.*—Reagent grade, halogen-free, sp. gr. 1.50.

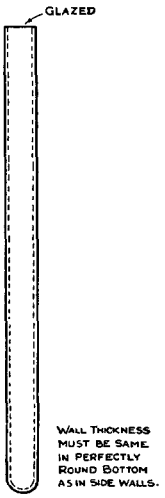
(b) *Silver nitrate.*—Reagent grade, powd.

* To alter the conditions (temp., size of sample, vol. of acid, etc.) might prove to be dangerous, presenting an explosion hazard.

APPARATUS

(a) *Combustion tubes.*—Use clean, 240 ±10 mm long by 13 ±0.7 mm O.D. standard wall Pyrex tubes or 210 ±10 mm long by 13 ±0.8 mm O.D. Pyrex tubes with 2.3 ±0.3 mm walls (see Table), free from flaws and with a rounded seal at the bottom. (See Figure.)

Combustion Tube	*Wall Thickness mm*	*O.D. mm*	*Length mm*	*Length of Sealed Tube between Bottom and Start of Taper at Shoulder mm*	*Vol. HNO_3 (Sp. Gr. 60°F., Approximately 1.5) ml*	*Temp. °C.*
Heavy-walled	2.3 ±0.3	13 ±0.8	210 ±10	150 to 175	0.5	250
Thin-walled	1.2 ±0.2	13 ±0.7	240 ±10	180 to 210	0.3	300



COMBUSTION TUBE

(b) *Furnace.*—Elec. with capacity of 4 or more tubes held at an angle of ca 45°. Furnace must maintain temp. of 250 ±10 or 300 ±10°C. for 5 or more hrs, with no more than 5°C. difference between any 2 points on a tube or 5° difference between similar points on any 2 tubes. Furnace must have variable resistor or other device to adjust furnace to desired temp. Open end of furnace wells must have safety device to retain glass in furnace should tube explode, and device must be provided for removing individual tubes from wells.

(c) *Filter tubes.*—Micro filter tube with medium coarse porosity (av. pore diam. 15–25 μ), fritted disc and capacity of 3 ml.

SAMPLE

Using a microchemical balance, weigh 5–20 mg sample contg a min. of 1.5

mg Cl or 2.5 mg Br or using a semimicrochemical balance, weigh 10–20 mg sample contg a min. of 2.5 mg Cl or 4.5 mg Br.

Solid samples.—Weigh by difference in charging tube.

Viscous liquids or gummy solids.—Weigh in porcelain boat.

Volatile liquids.—Weigh in 5 cm sealed glass tube, 1–2 cm I.D. with capillary tip. Break off tip of the capillary before placing in combustion tube sealed end down.

DETERMINATION

Place weighed sample in combustion tube, add powd. $AgNO_3$ 100% in excess of amount estimated to be necessary, and add 0.3 ± 0.03 or 0.5 ± 0.05 ml fuming HNO_3 depending on type of combustion tube (see Table). Using blast lamp and holding tube at 30–40° angle, seal tube at a distance from bottom so that sealed tube will have length shown in Table. Rotate tube slowly in flame until wall thickens, pull out and seal off narrow neck of tube. Wall of seal should not be less than ¾ the thickness of tube wall. (If sample and HNO_3 react at room temp., immediately cool bottom of tube in ice H_2O or dry ice-acetone bath). Immediately place tube in furnace. Heat tubes for 5 hrs at 250 or 300 ± 10°C. (see Table).

Observe the following precautions before and during opening of combustion tubes: (a) Place asbestos glove on hand used to hold small burner or hand torch; (b) protect face by transparent face mask or work behind safety shield; (c) be certain tube has cooled to room temp.; (d) force tip of tube ca 2″ out of furnace well; (e) gently flame end to drive all acid from tip and upper walls; and (f) soften tip with small hot flame until pressure in tube is released by blowing out softened glass.

Remove vented tube from furnace and cut off constricted end by scratching tube with file ca ½″ from shoulder of open end, moisten scratch and touch with tip of very hot glass rod. Remove end of the tube with care and fire polish to avoid contaminating ppt with glass splinters.

Rinse walls of tube with distd H_2O until tube is ca ¾ full, place in steam or boiling H_2O bath, protected from light, and digest until ppt is coagulated (ca 30 min.). Place previously washed, dried and weighed filter tube in one-holed stopper in suction flask, connect short arm of siphon tube to filter tube thru small rubber stopper and adjust Carius tube so that long arm of siphon almost touches the ppt. Transfer ppt to filter tube by suction. Rinse tube and ppt alternately with 1% HNO_3 and 95% ethanol using 2 or 3 ml portions for each rinse.

Remove siphon, rinse tip and stopper with alcohol and rinse filter tube and ppt first with acid, then with alcohol. Wipe outside of filter tube with moist chamois (or cheesecloth) and dry at 125°C. for 30 min. in air oven or 80°C. for 30 min. in vacuum oven, cool to room temp. (30 min.), and weigh. Handle dry tube with chamois finger cots or tweezers. Make blank run and subtract any correction from wt of sample ppt.

CALCULATIONS

$$\frac{(\text{wt ppt} - \text{blank}) \times \frac{\text{Cl}}{\text{AgCl}}}{\text{wt sample}} \times 100 = \% \text{ Cl}$$

$$\frac{(\text{wt ppt} - \text{blank}) \times \frac{\text{Br}}{\text{AgBr}}}{\text{wt sample}} \times 100 = \% \text{ Br}$$

SELECTED REFERENCES

Anal. Chem. **21**, 1555 (1949); **23**, 1689 (1951).

STEYERMARK, AL, "Quantitative Organic Microanalysis," Blakiston, 1951, p. 184.

NIEDERL, J. B. and NIEDERL, V., "Micromethods of Quantitative Organic Analysis," 2nd Ed., Wiley, 1942, p. 279.

(2) The following Carius and catalytic combustion methods with titrimetric determination of sulfates was adopted, first action:

CARIUS COMBUSTION METHOD

REAGENTS

(a) *Fuming nitric acid.*—Reagent grade, sp. gr. 1.50.

(b) *Sodium chloride.*—Reagent grade, fine crystals.

(c) *Barium chloride solution.*—Ca 0.02 *N*, standardized by titrating 5–7 mg freshly dried K_2SO_4, A.C.S. specification (weighed to the nearest 0.01 mg) by same procedure used for the sample titration. Correct titration for indicator error by blank run.

(d) *Potassium sulfate.*—A.C.S., powd. and dried.

(e) *Sodium hydroxide.*—Ca 0.1 *N*.

(f) *Hydrochloric acid.*—Ca 0.02 *N*.

(g) *Phenolphthalein.*—0.5% soln in 50% alcohol.

(h) *Sulfate indicator.*—"T.H.Q." prepd indicator. (Betz Laboratories, Philadelphia, Pa.)

APPARATUS

(a) *Combustion tubes and furnace.*—See app. in Carius combustion method for Br and Cl, item (1) above.

(b) *Titration assembly.*—5 ml buret graduated in 0.01 ml; rectangular titration cell ca 2×4×5 cm with min. capacity of 50 ml; and standard orange-red glass color filter having 37% transmittancy at 550 mμ. (Arthur H. Thomas Co., Philadelphia, Pa., Cat. No. 9324-H.) Cell and filter are placed side by side on milk glass window illuminated from below, preferably by fluorescent light. The light source must be masked so that only the cells and filter are illuminated.

SAMPLE

Using microchemical balance, weigh 5–20 mg sample contg not less than 0.75 mg S or using semimicrochemical balance, weigh 10–20 mg sample contg not less than 0.75 mg S. Weigh samples as indicated in Carius combustion method for Br and Cl, item (1) above.

DETERMINATION

Place weighed sample in combustion tube, add NaCl 100% in excess of amount equivalent to S in sample, and proceed as directed under "Determination" in Carius combustion method for Br and Cl, item (1) above, beginning "and add . . . fuming HNO_3 . . . " thru end of third paragraph " . . . with glass splinters."

Transfer contents of tube to 50 ml beaker, rinsing tube 4–6 times each with 3–5 ml H_2O. Evap. to dryness on steam bath.

*Volumetric Determination.**—Dissolve residue in 10 ml distd H_2O. Pour soln into titration cell, add 1 drop phenolphthalein indicator, make just alk. with 0.1 *N* NaOH, then acid with 0.02 *N* HCl adding one drop in excess. Add ca 0.15 g of "T.H.Q." indicator, stir to dissolve, rinse beaker 2–3 times using sufficient ethanol so that final soln is ca 50% ethanol. Titrate with standard $BaCl_2$ soln from 5 ml buret ml until stable color of the soln immediately after stirring matches standard glass color filter. (Make certain end point taken is real and not pseudo end point which will fade on standing 1–2 min.) Run blank on reagents and correct titration value.

CALCULATION

$$\frac{(\text{ml } BaCl_2 - \text{blank}) \times N \times 16.033 \times 100}{\text{Sample wt (mg)}} = \% \text{ S.}$$

* Volumetric detn cannot be used if sample contains phosphorus.

CATALYTIC COMBUSTION METHOD

REAGENTS

Use reagents of the Carius combustion method above, and

(a) *Bromine water.*—Satd aq. soln of Br stored in glass-stoppered bottle.

APPARATUS

(a) *Oxygen supply.*—Use O pressure cylinder with 2 stage reducing valve having needle valve control on low pressure side, or any other source which will supply pure O at 12–15 ml/min.

(b) *Purification train.*—If O is not free from S contg gases, purify by passing gas through a tube containing first dehydrite then ascarite.

(c) *Combustion tube.*—Quartz (or Vycor) with dimensions shown in Figure.

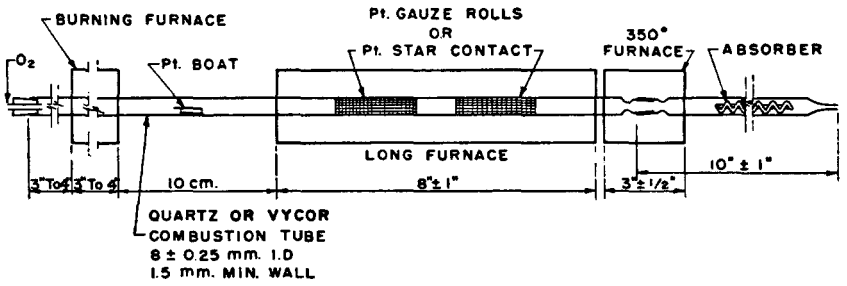
(d) *Absorber.*—Beasley type spiral connected to combustion tube by ground joint.

(e) *Catalyst.*—2 Pt star contacts or 2 Pt gauze rolls made from 5 cm squares of ca 50 mesh gauze. Rolls to have diam. within 1 mm of I.D. of combustion tube.

(f) *Furnaces.*—Elec. or gas, elec. preferred, both providing temp. inside combustion tube of at least 750°C. and preferably 800°C. or over. Sample burner operated mechanically or manually, the former being preferred. Short furnace for ground joint, preferably elec., to operate at ca 350°C. Rate of motion of sample burner 0.5 cm/min.

Titration assembly.—See Carius combustion method above.

COMBUSTION ASSEMBLY FOR SULFUR DETERMINATION BY CATALYTIC METHOD



SAMPLE

Use sample described under Carius combustion method above.

DETERMINATION

Clean catalysts by boiling 10 min. in ca 6*N* HNO_3 and flaming over non-luminous flame, using Pt tipped tweezers. Place catalysts in combustion tube and set tube in furnaces as shown in Figure, heat long furnace to at least 750° and preferably 800°C. or over.

Moisten entire spiral of absorber by drawing into it with gentle suction 5–10 ml of Br water. Care must be exercised to keep ground joint dry. Drain excess soln from absorber and attach to combustion tube with ground joint in 350° furnace.

Place sample in combustion tube 5 cm from long furnace, connect O source and adjust flow to 12–15 ml/min. using flow meter or calibrated bubble counter.

Heat sample burner to at least 750°, preferably 800°C. or over, bring to 5 cm from sample and move over sample area at rate of 0.5 cm/min. (burning time 20 min.). Continue sweeping with O for 10 min. (total combustion time 30 min.). Disconnect absorber, allow joint to cool 3–5 min.

*Volumetric determination.**—Rinse contents quantitatively into 50 ml Erlenmeyer flask using 15–20 ml H_2O. Rinse outside of absorber tip. Add 5 drops Br water, boil until Br is dispelled and cool under tap. Continue as under Carius combustion method beginning with "Pour solution into titration cell, . . . "

38. RADIOACTIVITY

No additions, deletions, or other changes.

39. STANDARD SOLUTIONS

(1) The official method for standardization of hydrochloric acid, constant boiling method, **39.11** (p. 758), was revised as follows and adopted, first action:

Dil. 850 ml analytical-reagent grade HCl (35–37% HCl) with 750 ml H_2O. Check sp. gr. with spindle and adjust to 1.10. Place 1500 ml in 2-liter flat-bottom distg flask, add ca 10 carborundum crystals (ca 20 mesh) and connect to long, straight inner-tube condenser. Heat on elec. hotplate and distill at rate of 5–10 ml/min., keeping end of condenser open to air. When 1125 ml has been distd, change receivers and catch next 225 ml, which is constant boiling HCl, in Erlenmeyer flask with end of condenser inserted into flask but not below surface of liquid. Read barometer to nearest mm at beginning and end of collection of 225 ml portion and note barometer temp. Average readings.

Calc. air wt in grams (G) of this constant boiling HCl required to give one equivalent wt of HCl from the following equation (formula is applicable to pressures of 540–780 mm Hg):

$$G = \frac{P_0 + 7680}{46.839}$$

P_0 = barometric pressure in mm Hg corrected to 0°C. for expansion of Hg and of barometer scale. For brass scale barometer the following correction is sufficiently accurate:

$$P_0 = P_t(1 - .000162t)$$

where t = temp. of barometer in °C.

Weigh out required quantity of constant boiling HCl in tared, stoppered flask with accuracy of at least one part in 10,000. Dil. immediately and finally make to vol. with CO_2-free H_2O at desired temp.

40. VITAMINS

(1) The first action chemical method for nicotinic acid, *This Journal*, **34,** 99 (1951) as revised, *ibid*, **35,** 102 (1952) was adopted as official.

(2) The following method for assay of vitamin B_{12} feed supplements ranging from approximately 1.0 mg to 10 mg vitamin B_{12} per pound was adopted, first action:

* Volumetric detn. cannot be used if sample contains phosphorus.

REAGENTS

(a) *Standard cyanocobalamin stock solution.*—To a suitable quantity of U.S.P. cyanocobalamin reference standard, accurately weighed, add sufficient 25% alcohol to make a soln, each ml to contain 1.25 mmg of cyanocobalamin. Store in a cool place and use no longer than 60 days.

(b) *Standard cyanocobalamin solution.*—To 10 ml of standard cyanocobalamin stock soln (a) add 25 ml 1.0% $NaHSO_3$ in *p*H 4.5 phosphate-citrate buffer. Autoclave for 15 min. (121–123°C.) and dil. with H_2O to 500 ml. Dil. an aliquot of this soln 1000 times. Each ml represents 0.025 millimicrogram of cyanocobalamin. Prepare a fresh standard soln for each assay.

(c) *Basal medium stock solution.*—To prevent the possible formation of colloidal suspensions or ppts which may result in either slow growth or high blanks (or both) the following sequence of ingredient additions should be followed:

100 mg l-cystine and 100 mg d,l-tryptophane dissolved in ca 10 ml 1 *N* HCl.	
Adenine-guanine-uracil soln, (f)	5 ml
Xanthine soln, (g)	5 ml
Vitamin soln I, (h)	10 ml
Vitamin soln II, (i)	10 ml
Salt soln A, (j)	5 ml
Salt soln B, (k)	5 ml
Asparagine soln, (e)	5 ml
Acid-hydrolyzed casein soln, (d)	25 ml
Dextrose, anhyd.	10 g
5 g Sodium acetate, anhyd. and 1 g ascorbic acid dissolved in 50 ml H_2O	
Polysorbate 80 soln, (l)	5 ml
Adjust to *p*H 6.0 with NaOH soln, and finally add H_2O to 250 ml.	

(d) *Acid-hydrolyzed casein solution.*—Mix 100 g vitamin-free casein with 500 ml dil. HCl (1 in 2) and reflux the mixture for 8–12 hrs. Remove the HCl by distn under reduced pressure to a thick paste. Redissolve paste in H_2O, adjust *p*H to 3.5 ($\pm$0.1) with NaOH soln and add H_2O to 1000 ml. Add 20 g activated charcoal, stir 1 hr, and filter. Repeat twice this treatment with activated charcoal. Store under toluene in refrigerator at a temp. not below 10°C. Filter soln if a ppt forms upon storage.

(e) *Asparagine solution.*—Dissolve 2.0 g l-asparagine in H_2O to make 200 ml. Store under toluene in a refrigerator.

(f) *Adenine-guanine-uracil solution.*—Dissolve 0.2 g each adenine sulfate, guanine hydrochloride, and uracil with the aid of heat, in 10 ml of 20% HCl, cool, and add H_2O to make 200 ml. Store under toluene in a refrigerator.

(g) *Xanthine solution.*—Suspend 0.2 g xanthine in 30–40 ml H_2O, heat to ca 70°, add 6.0 ml of NH_4OH (40 ml dild to 100 ml with H_2O), and stir until solid is dissolved. Cool, and add H_2O to 200 ml. Store under toluene in a refrigerator.

(h) *Vitamin solution I, riboflavin-thiamine-biotin-nicotinic acid solution.*—Prep. a soln in 0.02 *N* acetic acid, each ml to contain 25 mmg riboflavin, 25 mmg thiamine hydrochloride, 0.2 mmg biotin, and 50 mmg nicotinic acid. Store, protected from light, under toluene in refrigerator.

(i) *Vitamin solution II, p-aminobenzoic acid-calcium pantothenate-pyridoxine-pyridoxal-pyridoxamine-folic acid solution.*—Prep. a soln in 25% neutralized alcohol, each ml to contain 50 mmg p-aminobenzoic acid, 25 mmg ca pantothenate, 100 mmg pyridoxine HCl, 100 mmg pyridoxal HCl, 20 mmg pyridoxamine dihydrochloride, and 5 mmg folic acid. Store in refrigerator.

(j) *Salt solution A.*—Dissolve 10 g KH_2PO_4 and 10 g K_2HPO_4 in H_2O to make 200 ml. Add 2 drops HCl and store under toluene.

(k) *Salt solution B.*—Dissolve 4.0 g $MgSO_4 \cdot 7H_2O$, 0.2 g NaCl, 0.2 g $FeSO_4 \cdot 7H_2O$, and 0.2 g $MnSO_4 \cdot H_2O$ in H_2O to make 200 ml. Add 2 drops HCl, and store under toluene.

(l) *Polysorbate 80 solution.*—Dissolve 20 mg of polysorbate 80 in sufficient alcohol to make 200 ml. Store in refrigerator.

(m) *Culture medium.*—Dissolve 0.75 g water-soluble yeast extract, 0.75 g peptone, 1 g anhyd. dextrose, and 0.2 g KH_2PO_4 in 60–70 ml H_2O. Add 10 ml tomato juice prepn (n), and 1 ml polysorbate 80 soln. Adjust to *p*H 6.8 with NaOH soln, and add H_2O to 100 ml. Place 10 ml portions in test tubes, and plug with cotton. Sterilize tubes and contents in an autoclave for 15 min. at 121–123°C. (exhaust line temp.). Cool as rapidly as possible to avoid color formation from overheating.

(n) *Tomato juice preparation.*—Centrifuge 3000 ml commercially canned tomato juice. Suspend 10–20 g analytical filter-aid in the supernatant liquid and filter with suction thru a layer of analytical filter-aid of sufficient thickness so that a clear, straw-colored filtrate is obtained.

(o) *Suspension medium.*—Dil. measured vol. of basal medium stock soln with an equal vol. H_2O. Place 10 ml portions of the dild medium in test tubes. Sterilize, and cool as directed for the culture medium (m).

(p) *Stock culture of the Lactobacillus leichmannii.*—To 100 ml culture medium add 1.0–1.5 g agar, and heat with stirring on steam bath until the agar dissolves. Add ca 10 ml portions of the hot soln to test tubes, plug with cotton, sterilize for 15 min. in autoclave at 121°–123° (exhaust line temp.), and allow tubes to cool in upright position. Prep. stab cultures in 3 or more tubes, using pure cultures of Lactobacillus leichmannii.† (Before using a fresh culture in this assay, make at least 10 successive transfers of the culture in a two-week period.) Incubate 16–24 hrs at any selected temp. between 30° and 37° but held constant to within ±0.5°, and finally store in refrigerator.

The activity of the microorganism can be increased by daily, or twice-daily transfer of the stab culture, and may be considered satisfactory when definite turbidity in the liquid inoculum can be observed 2–4 hrs after inoculation. With a slow growing culture a suitable response curve is seldom obtained, and may give rise to erratic results.

(q) *Inoculum.*—Make transfer of cells from stock culture of Lactobacillus leichmannii to sterile tube contg 10 ml culture medium. Incubate for 16–24 hrs at any selected temp. between 30° and 37° but held constant to within ±0.5°. Under aseptic conditions, centrifuge culture, and decant supernatant liquid. Suspend cells from culture in 10 ml sterile suspension medium, centrifuge, and decant supernatant liquid. Again suspend cells in 10 ml sterile suspension medium, centrifuge, and decant supernatant liquid. Repeat this process a third time. Finally resuspend cells in 10 ml sterile suspension medium, add 1 ml of this suspension to 10 ml of sterile suspension medium, and mix. The cell suspension so obtained is the inoculum.

TEST SOLUTION OF THE MATERIAL TO BE ASSAYED

Autoclave ca 1 g or 1 ml of sample, accurately measured, for 15 min. (121–123°C.) in 25 ml of 0.1 *M* phosphate-citrate buffer at *p*H 4.5 (4.54 ml 0.2 *M* Na_2HPO_4 plus 5.46 ml 0.1 *M* citric acid) contg 1.0% freshly prepd $NaHSO_3$. Allow any undissolved particles to settle, or centrifuge if necessary. Dil. an aliquot of the clear soln with H_2O so that the final test soln contains a vitamin B_{12} activity equivalent to ca 0.025 millimicrogram of cyanocobalamin.

The amount of bisulfite in the assay soln should not exceed 0.025 mg/ml or the assay tube should not contain more than 0.125 mg.

† Pure cultures of Lactobacillus leichmannii may be obtained from the American Type Culture Collection, 2029 M Street, N.W., Washington, D. C. as No. 7830.

DETERMINATION

Because of the extremely high biological activity of the cobalamins the cleaning of glassware is *highly important.*

To 20×150 mm test tubes, add *in triplicate,* 0.0 ml, 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml, respectively, of the standard cyanocobalamin soln. To each tube add 5.0 ml basal medium stock soln and sufficient H_2O to make 10 ml.

To similar test tubes add *in triplicate* respectively, 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, and 5.0 ml of the test soln of material to be assayed. To each tube add 5.0 ml of basal medium stock soln and sufficient H_2O to make 10 ml.

Mix, cover the tubes suitably to prevent bacterial contamination, and sterilize tubes and contents in autoclave for 5 min. at 121–123°C. (exhaust line temp.) arranging to reach this temp. in not more than 10 min. Cool as rapidly as practicable to avoid color formation from overheating. Take precautions to maintain uniformity of sterilizing and cooling conditions thruout the assay.

Sterilization of the assay tubes is critical. During heating cyanocobalamin, and perhaps other analogues, are converted to an analogue of the vitamin that is completely available to the organism. The 5 min. sterilization time is sufficient for this purpose, and to assure adequate sterilization. Longer heating periods cause excessive browning which may inhibit the growth of the organism. It is important to use a sterilizer that can be brought to temp. in a short time and which gives uniform heating. Too close packing of tubes in the autoclave, or over-loading, may cause variation in heating rate. Identical conditions for the cooling of tubes should be maintained.

Aseptically inoculate each tube with 1 drop of inoculum, and incubate for 16–24 hrs at any temp. between 30° and 37°C. but held constant to within ±0.5°C. Read turbidity of the tubes in a suitable instrument at a wavelength of 660 mμ after thoro shaking. Allow ca the same time interval to elapse prior to each tube reading.

Disregard results of an assay in which contamination with a foreign organism is evident or in which the control tubes of the standard series, to which no standard cyanocobalamin soln has been added, give a reading less than 85% transmission (preferably less than 90%), when read against an H_2O blank, which indicates interference due to vitamin B_{12} activity in the basal medium stock soln or inoculum.

CALCULATION

Prepare a standard concn response curve by plotting the galvanometer readings for each level of the standard cyanocobalamin soln used, against millimicrograms of cyanocobalamin contained in the respective tubes. Draw the smooth curve which by visual inspection appears to fit best the plotted points. From this standard curve, det. by interpolation for each tube the amount of cyanocobalamin equivalent to the vitamin B_{12} activity of each ml of the test soln of the material to be assayed.

Since in microbial assays occasional inexplicable aberrant values are obtained in individual tubes, inspect the series of values and set aside any which vary markedly from most of the series. Strike a provisional average of the remaining values, and set aside any of the latter which are less than 90% and more than 110% of the provisional av. If less than 10 of the 15 original values remain, the data are insufficient for calcg the potency; if 10 or more values remain, calc. the potency from the av. Disregard any assay in which the calcd potency is less than 75% or more than 125% of the assumed potency.

ERRATA AND EMENDATIONS, METHODS OF ANALYSIS, A.O.A.C., 1950

The errors that are reported from time to time by those using this book will be published in *This Journal*. The following changes should be noted.

Section	*Page*	
6.67	115	Change title to read "MIXED CAROTENES IN FRESH PLANT MATERIALS BY PHASIC SEPARATION (*27*)—FIRST ACTION."
6.67(a)	115	Delete second and third paragraphs. Add at end of first paragraph, "If soln contains chlorophyll, i.e., absorbs light in the region of 6600 Å, treat as indicated in **6.68(3)**. Det. carotenes as directed in **40.8**."
13.11(e)	194	Delete "Molar."
16.28, last line, par. 1	283	Add "using **33.49(a)** for the detn."
16.33, line 2	284	Change "**18.16**" to "**18.15–18.17.**" (Supplementary correction.)
21.14 first line under formula	341	Delete "normal soln" after "P" and "I" definitions.
21.15	341	Change "1.672" in formula to "1.267."
22.63, line 10	357	Change "1.5" to "15."
31.14(c), line 3	538	Change "**(a)**" to "**(b).**"
40.9(a), last line	771	Change "**6.67(a)**, (1) or (2)" to "**40.8.**"
40.9(b)	771	Change title to read "*Alternative extraction procedure for fresh materials or silages* (*6*)."

NOTE: The report on "Changes in Methods" should also be consulted.

ERRATA FOR AUGUST 1952 JOURNAL

In the report on Enzymes, by J. W. Cook, change the letter "B" to "C" in the following places in the text of the method, to avoid confusion with the reagent designated as "(d) Test Paper B."

(1) page 547, first paragraph, last line;
(2) page 548, Table 1, third column (throughout the column);
(3) page 549, first paragraph under "Discussion," last line;
(4) page 549, last paragraph, second and third lines from bottom.

REPORT OF THE SECRETARY-TREASURER

K. L. MILSTEAD

The Executive Committee was called to order by President Lepper at 2:00 P.M., on Sunday, Sept. 28, 1952, in the West Room of the Shoreham Hotel. All members except H. A. Halvorson were present. The audit of the accounts of the Association as reported by John W. Bisselle and Company was presented and accepted. The statement of the financial condition of the Association follows:

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, INC.

BALANCE SHEET—SEPTEMBER 24, 1952

ASSETS

Current Assets:		
Cash, Lincoln National Bank		$ 34,627.60
Cash on hand		670.20
Office cash fund		15.70
Accounts receivable		5,029.48
Accrued interest receivable		250.00
Inventories		15,842.26
Total Current Assets		$ 56,435.24
Fixed Assets		
Furniture and fixtures		1,669.86
Investments		52,689.00
Total Assets		$110,794.10

LIABILITIES

Current Liabilities:		
Accounts payable		$ 3,896.27
Social security tax deductions		33.60
Withholding tax collections		132.48
Total Current Liabilities		$ 4,062.36

SURPLUS

Balance, September 27, 1951	$100,302.08	
Add net income for the fiscal year ended September 24, 1952	6,429.66	
Balance, September 24, 1952		106,731.74
Total Liabilities and Surplus		$110,794.10

The Executive Committee recommended that a portion of the surplus cash be invested in government bonds.

It is recommended that Dr. Reindollar be reappointed as Chairman of the Committee on Recommendation of Referees.

One vacancy occurs this year on each of committees A, B, C, and D, and it is recommended that the following members be appointed to fill these vacancies—Chester D. Tolle, Jonas Carol, Frank A. Vorhes, Jr., and R. A. Osborn.

The Executive Committee recommends that a committee of five be appointed by the President on Terminology and Application of Spectrophotometric Methods, who will cooperate with other interested scientific groups in the interest of uniformity.

The Association has been invited to become a Public Member of The Food Law Institute. The Executive Committee recommends that the Association accept this invitation. It carries no financial responsibilities and is simply advisory in character. The following resolution is recommended for adoption:

Whereas, The Food Law Institute was created to promote and disseminate knowledge of the food and associated laws through research and education and to inform the public, professions, universities, and industry of the profound social, economic, and legal significance of these laws; and

Whereas, The Association of Official Agricultural Chemists was created to provide the scientific information and techniques necessary for the full realization of the objectives of those laws; and

Whereas, This Association is in accord with the research and educational objectives of The Food Law Institute: Now, Therefore,

Be It Resolved, That the Association of Official Agricultural Chemists join The Food Law Institute as a Public Member.

Approved.

REPORT OF THE COMMITTEE ON NECROLOGY

HARRY SCHARER

The untimely death of Harry Scharer on May 27, 1952 was a shock to all who knew him. From 1926 to 1951 he had served as chemist in the Bureau of Food and Drugs of the New York City Department of Health, analyzing foods and drugs and developing simplified testing procedures for such analyses. He was Assistant Director of Research of the Applied Research Institute from 1940 to 1942 and Director of Research from 1942 to 1952. From 1947 to 1952 he was consultant to the U. S. Public Health Service. During his short career, Mr. Scharer gave papers before meetings of the International Association of Milk Dealers, the International Association of Milk and Food Sanitarians and the International Association of Ice Cream Manufacturers. In 1942–1943, he was Nominee for the Borden Award for Research in Dairy Chemistry.

Harry Scharer had a flare for simplification, as shown by his tendency to make all tests as simple as possible for technicians, not only for the purpose of saving time and labor, but primarily for the purpose of eliminating

error. Thus, by concentrating into a tablet form a test which might otherwise require a series of processes, he enabled technicians to make tests on milk and water by simple processes instead of lengthy complicated procedures.

The Scharer Phosphatase Test which he developed has been accepted as a procedure for the examination of both milk and cream, not only by health authorities throughout the country, but also by the U. S. Army, U. S. Navy, and U. S. Air Force Procurement Agencies, whose specifications include it as their official test.

Harry Scharer will be remembered for his flare for simplification of testing procedures, his clear analytical thinking in scientific fields, and his high regard for integrity.

PHILIP H. SMITH

Mr. Smith was born on September 14, 1876, and, following a long illness, died on May 10, 1952. Between these dates Mr. Smith's business life was concerned almost entirely with feed control work. He entered the Massachusetts Foods and Feeding Laboratory in 1900 and continued his work there until his retirement in 1946. He became Chemist in Charge of the feed laboratory in 1907, and from 1939 to 1946 he was head of the feed, fertilizer and seed control service.

DR. LESLIE C. HARLOW

Dr. Leslie Harlow, born in Shelburne, N. S., 1872, died January 16, 1952. He was Provincial Chemist and Professor of Chemistry, Nova Scotia Agricultural College, Truro, N. S. (retired).

Dr. Harlow was graduated from Cornell University, B.S.A. (1899), B.Sc. (1900), and in 1906 became Professor of Chemistry at the Nova Scotia Agricultural College, where he taught until 1941. He was a pioneer in experimentation with ground limestone and a promoter of its use, and was a strong advocate of the soil building program, for which he was awarded D.Sc. (Hon.) by Acadia University. For many years Dr. Harlow was an Official Analyst for the Federal Department of Agriculture under the Fertilizer and Pest Control Products Acts.

He was a Fellow of the Agricultural Institute of Canada, a Fellow of the American Association for the Advancement of Science, and a member of the Chemical Institute of Canada.

A. VALIN

Mr. Valin, at the time of his retirement in 1946, was Chief Dominion Analyst, Department of National Health and Welfare, Ottawa, Canada.

In 1901 he entered the Canadian government service, where he rose to be Superintendent of Laboratory in the Montreal offices of the Depart-

ment. His work on foods and drugs led to the establishment of many standards for these products. In May, 1945, Mr. Valin was transferred to Ottawa to head up the laboratory services of the Health Department. A quiet, friendly man, he enjoyed retirement at his home in New Edinburgh, Ottawa, until his death in April, 1951.

JOHN T. KEISTER

John T. Keister, born September 25, 1874, passed away on June 25, 1952. He received his Bachelor's and Master's degrees in Science from Virginia Polytechnic Institute, and, in 1907 a degree in Pharmacy from George Washington University. On January 1, 1904 he entered the Bureau of Chemistry as a Scientific Aid, and retired December 31, 1942.

During the early years of his long service in the Federal Government John Keister specialized in dairy products and became an expert in that subject. In later years his knowledge was utilized in the drafting of Federal Specifications and in the testing of samples in connection with the purchase of food supplies by the Veterans Administration and other governmental agencies. He was a quiet and deeply religious man with a friendly attitude toward his fellowmen.

J. R. ADAMS
J. F. FUDGE
JONAS CAROL, *Chairman*

REPORT OF THE COMMITTEE ON NOMINATIONS

Your committee proposes the following nominees and moves their election to the respective offices, as designated:

President, H. J. Fisher, Connecticut Agricultural Experiment Station, New Haven, Conn.

Vice President, E. L. Griffin, Production and Marketing Administration, Livestock Branch, USDA, Washington, D. C.

Secretary-Treasurer, William Horwitz, Food and Drug Administration, Federal Security Agency, Washington, D. C.

Secretary-Treasurer Emeritus, W. W. Skinner, Kensington, Md.

As additional members of the Executive Committee: W. F. Reindollar, Baltimore, Md.; K. D. Jacob, Beltsville, Md.; M. P. Etheredge, State College, Miss.; and Henry A. Lepper, Past President, Washington, D. C.

ALEX P. MATHERS
BRUCE POUNDSTONE
W. H. MACINTIRE, *Chairman*

Approved.

REPORT OF THE COMMITTEE ON RESOLUTIONS

Whereas, The success of this Association during the past year is due in large part to the painstaking efforts of our officers who have faithfully carried out their duties and responsibilities and have thoughtfully planned and efficiently conducted this, the 66th Annual Meeting of this organization: Now, Therefore,

Be it Resolved, That we express our earnest appreciation to President H. A. Lepper, Vice-President H. J. Fisher, and Secretary-Treasurer K. L. Milstead for their faithful service.

Whereas, The chief objective of this organization is to promote the development of reliable analytical methods and their acceptance for regulatory purposes and for general scientific use; and

Whereas, The attainment of these objectives is possible only through the exacting work of the Committee on Recommendations of Referees, the members of the several other committees, our Referees, Associate Referees, and collaborating analysts: Now, Therefore,

Be it Resolved, That we express our appreciation to these workers for having given of their time and energy to this work.

Whereas, The Association was honored by the presentation of a most instructive address by a distinguished speaker: Now, Therefore,

Be it Resolved, That we express our thanks to the Director, School of Nutrition, Cornell University, L. A. Maynard for appearing before us.

Whereas, It is an inspiration to us all when an authority speaks to us on the legal aspects of our work: Now, Therefore,

Be it Resolved, That we express to Charles Wesley Dunn, President of the Food Law Institute, Inc. our sincere thanks for addressing us.

Whereas, The results of our efforts are largely embodied in the Book of Methods and our Journal, the preparation of which are the results of the work of a small group of our members: Now, Therefore,

Be it Resolved, That we express to the members of the Editorial Board and to those who assisted them our appreciation of their efforts in accomplishing these difficult assignments.

Whereas, An esteemed colleague, who, through the years has rendered this association distinguished service as officer and in other capacities, is prevented by illness from attending this meeting, the first absence in 45 years: Now, Therefore,

Be it Resolved, That we extend to W. W. Skinner our sincere regards and our earnest wish for his speedy recovery in order that he may again join us in our deliberations.

Whereas, The success of our work and the acceptance of our methods depend upon the active participation of workers in the many government agencies and commercial institutions in the United States and Canada: Now, Therefore,

Be it Resolved, That we thank those organizations which have made it possible for so many individuals to participate actively in research on the development of methods and to participate in this meeting.

Whereas, The success of this meeting has depended upon adequate assembly rooms and facilities: Now, Therefore,

Be it Resolved, That this Association request its Secretary to express to the management of the Shoreham Hotel the thanks of our Association for making available rooms and other facilities, and for the many courtesies shown our members during this annual meeting.

H. H. HOFFMAN
C. F. SNYDER
E. W. CONSTABLE, *Chairman*

Approved.

CONTRIBUTED PAPERS

SEROLOGICAL METHODS IN THE REGULATORY CONTROL OF FOODS*

By ELIZABETH J. OSWALD (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

The general excellence of food products today is a tribute to the diligence of many in the field of food processing and food technology. Compared to the vast amount of material that is handled and evaluated before it reaches stores and eventually kitchens, the irregularities that are encountered are few, and chemical, physical or bacteriological means usually are adequate in the detection of these.

There is, however, at least one situation where none of the above mentioned tests apply and it is with this situation that we are concerned—namely the substitution or adulteration of a food, with another visually identical food. For example, there is no visual distinction between pieces of muscle tissue taken from a cow and a horse and placed side by side on a plate. Characterization of the pieces of tissue might be made chemically, but the procedure is time consuming and usually inconclusive. Identification can be made quickly and simply by resorting to the techniques of the immunologist.

Immunology, or the science of immune behavior, had interesting beginnings, and has, through the years, added immeasurably to our knowledge of the mechanics of disease. While many of the early immunologists actually used bacteria and bacterial products in their studies, others concerned themselves almost altogether with just the sort of differences discussed here, i.e., distinguishing between blood sera, tissues, and egg albumins of various species of animals. At this point, we can state quite simply the principle upon which all immunology is based. When a foreign protein substance or antigen is introduced into an animal's blood or tissue fluids, it reacts by producing in the blood soluble substances (antibodies) which combine specifically with the injected foreign material. This combination can occur in the animal body, or it can be demonstrated outside the animal body in a test tube. The phenomenon may manifest itself in several ways. The particular type of tube test with which we are concerned is the precipitin or "ring test." This reaction was first demonstrated by Rudolf Kraus (1) in 1897, when he showed that the contact or mixture of clear antisera with clear solutions of antigens gives rise to turbidities or precipitates. In 1899, Tchistovitch (2), in one of the earliest experiments along these lines, prepared anti-horse serum in rabbits by injecting the serum of the horse either into the muscle tissue of the animal, or directly

* Presented before the 66th Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, September 29, 30, and October 1, 1952.

into the abdominal cavity. After a period of several days, the rabbit was bled and the cellular elements of the blood removed, leaving the clear serum. This worker then found that by using the technique of Kraus—in other words by bringing into contact with diluted horse serum, the clear serum which he obtained from his treated rabbits, he obtained a clearly defined precipitate. He did not obtain such a precipitate when his serum was tested against the diluted serum of the ass, although other workers disagreed with this latter result. It is now known that the soluble proteins of closely related animals will cross-react. In general, these cross-reactions are noticeably less marked than the reaction which occurs with homologous antigens. It has also been found that cross-reactions can be obviated and sera rendered specific by absorption, a procedure which will be mentioned later.

Bordet (3), also in the year 1899, found that anti-hen serum reacted with pigeon serum as well as with hen serum, indicating the close relationship existing between the two species of fowl. Bordet and later Uhlenhuth (4) discovered that an animal injected with the blood serum of its own kind, or a species closely related to it, will either fail to produce antibodies at all, or will do so only feebly. This circumstance has been found useful in the study of animal relationships.

According to Nuttall in his book "Blood Immunity and Relationship" precipitin tests were used in the examination of food products as early as 1900. In the closing chapter he states that, "Uhlenhuth (4) (1900) found that the antiserum for the egg white of the fowl gave negative results with albuminous preparations which did not contain egg white; the contrary being the case where they contained egg white. The use of such antisera is therefore suggested where it is desired to prove the presence of egg white in prepared foods. Von Rigler (5) (1902) treated rabbits with honey, in the manner employed when they are immunified (immunized) with blood. The anti-honey serum only produced precipitation in dilutions of honey, not in those of grape or cane sugar. Normal rabbit serum had no such effect."

The field of immunology has, since those early days, expanded into an exceedingly complex branch of science. The basic facts still hold, however, and we have availed ourselves of them in the tests which are to be described.

Publicity associated with the horsemeat scandal in Chicago in 1950 made many states aware of meat industry irregularities which existed within their borders. It was requested that the Food and Drug Administration devise and run the necessary tests until the states were able to organize their own laboratory facilities. It has been on such a cooperative basis that testing has been done, with the Federal Government bearing the interim expense.

The test, dated April 22, 1952, which is currently used by the Food and

Drug Administration is the following. The method of preparing the antigen is adapted from the procedure of Proom (6).

PROCEDURE

Preparation of antisera.—The serum of the desired species (i.e., beef, horse, etc.) is used to prepare the antigen. Twenty-five ml of serum is diluted with 80 ml of distilled water. To this solution is added 90 ml of 10% potassium alum. The *p*H of this solution is adjusted to the isoelectric point specific to the alum-protein complex of the particular serum used (i.e. horse, *p*H 6.5; pork, *p*H 5.5, etc.) with 5*N* NaOH. The precipitate is twice washed by means of the centrifuge with 200 ml portions of 1:10,000 merthiolate saline. Finally, this precipitate is made up to a volume of 100 ml with 1:10,000 merthiolate saline.

Five ml of this antigen suspension is injected, intramuscularly, into each hind leg of a rabbit, or a total of 10 ml per rabbit, as the immunizing dose. The rabbits are given no food 24 hours prior to bleeding and are bled from 15 to 20 days after the immunizing injections.

The resulting antisera are tested for sensitivity and specificity. A satisfactory antiserum for the purpose of meat identification will give a strong positive result at a dilution of antigen of 1:1000 within twenty minutes at room temperature and will not cross react with antigens of other species diluted 1:50.

Standardization of antisera.—Once the antiserum has been prepared, it must be standardized for specificity to the raw antigen. This is done by setting up a dilution series of the raw antigen as well as similar dilution series of raw antigens of all the other meats that may be in the products to be tested. Thus, anti-horse serum would be tested against its own specific antigen and cross-tested against the antigens of beef, lamb, pork, etc., to determine if any cross-reaction takes place. When these cross-reactions occur, they can be negated by treating the antisera with a small amount of the antigen against which they cross react, followed by filtration or centrifugation to remove the resulting antigen-antibody precipitate.

PRECIPITATION TEST

(a) *Titration of antisera.*—Prepare serial dilutions of the antigen in physiological saline, ranging from 1:100 to 1:50,000 and place 1.0–2.0 ml amounts in a series of dry, clean 4 mm precipitation tubes. Smaller amounts can be used, but the pressure of the larger column at the interface of the antigen-antisera results in a more rapid appearance of the specific precipitate. Using a capillary pipette, introduce a small amount of the antiserum at the bottom of the tube in such a way that two distinct layers are formed. If the antigen and antiserum react, a precipitate appears as a white ring at the interface of the two solutions. The test is best viewed with artificial light against a black background.

At the same time, place a small amount of the serum in tubes containing the lowest dilution (1:50) of the other antigens, and determine whether a cross-reaction occurs with antigens other than the specific one against which the antiserum was prepared. If a precipitate does not develop against any of the antigens apt to be in the meat products to be tested, the antiserum can be assumed to act specifically with the antigen against which it was prepared.

(b) *Examination of unknown.*—Remove a 50 g portion of the material to be tested and homogenize in a Waring blendor with 100 ml of physiological saline. Filter through coarse filter paper to remove particulate matter and through a Seitz bacterial filter to obtain a perfectly clear filtrate. Place 1.0 ml of this filtrate in as many precipitation tubes as may be required to conduct the test (i.e., if the material is to be tested against anti-beef and anti-horse sera, prepare three tubes; the third tube is for a control using normal rabbit sera to make certain nothing in the extract

reacts with the sera as such). Add the appropriate antisera and observe as above. Certain processed meats such as wieners, luncheon meats, etc., have a high salt content which may give an extract with a specific gravity near that of the antisera, when prepared as above, and the two layers may tend to intermingle. To avoid this situation, all processed products are homogenized with 0.2% saline in lieu of physiological saline.

Since May 9, 1950, 1,113 tests have been run by the Food and Drug Administration on meat and meat products from fifteen states and eleven Food and Drug Administration Districts. Of this number, 226 samples have given a positive test with the specific horse antiserum, indicating an adulteration of the product with horsemeat. The distribution of these samples is given in the following table:

TABLE 1.—*Meat samples tested from May 1950 to June 1952*

STATES	NUMBER TESTED	NUMBER POSITIVE	DISTRICT	NUMBER TESTED	NUMBER POSITIVE
Arizona	1	1	Atlanta	7	5
Arkansas	281	28	Baltimore	6	2
Illinois	3	0	Boston	6	2
Indiana	25	14	Buffalo	1	0
Iowa	4	3	Chicago	16	14
Kentucky	5	0	Cincinnati	18	1
Missouri	49	3	Dayton (Cinn.)	6	0
New York	1	0	Miami (Atlanta)	7	7
North Carolina	21	10	New York	17	7
New Jersey	1	0	Philadelphia	3	3
Ohio	496	86	St. Louis	1	0
Oklahoma	46	3	Grand Total		
Rhode Island	64	36	1113 Total Samples Tested		
South Carolina	1	1	226 Total Positives		
Tennessee	27	0			

Two general types of statute infringement have been encountered during the course of this work. One has been the adulteration of beef products with horsemeat, or the outright substitution of the latter for beef. Another, which is a sort of borderline offense, involves the addition of pork to hamburger during the seasons when pork is less expensive than beef. This substitution is of considerable potential danger to the unsuspecting consumer, who, liking his hamburger rare, may become just one more trichinosis case history.

In the fall of 1951, the Food and Drug Administration became aware of a growing practice among seafood dealers in certain sections of the country of substituting fillets of one type of fish for those of another more expensive and popular variety. These fish, easily identifiable whole, were indistinguishable when cut into sections and frozen, so that except for

the relatively few dealers who recognized a slight visible difference in flesh texture, and the ultimate consumer, whose palate detected some taste aberration, the substitution usually went unnoticed. It was decided that an investigation was in order to determine whether or not serology could offer a solution to the problem. On October 16, 1951, samples of grouper and red snapper fish were received by the Division of Antibiotics for this preliminary work; and on April 25, 1952, the Food and Drug Administration approved the first recommendation for seizure of a lot of grouper fillets invoiced as red snapper. The test procedure, available in mimeographed form, follows in essence the procedure used in the identification of meat, except that the antigen used is dried fish flesh. Although only eight samples have been tested to date, resulting in one seizure, it is felt that such a test will have the effect of discouraging these substitution practices.

A third problem in food serology came from within the Administration itself. The Division of Food raised a question as to whether it would be possible to detect the presence of duck egg albumin in processed hen egg white. The presence of such traces of material, while not harmful per se, was felt to be indicative of irresponsible practices; and that, therefore, any means of detection could prove useful to a regulatory organization. Using techniques exactly similar to those which have been discussed, rabbit antiserum was obtained and absorbed with the heterologous egg albumin. With the absorbed anti-duck albumin serum it proved possible to detect as little as one part of duck egg albumin in 12,800 parts of hen egg albumin. It is possible, therefore, under conditions of practical usage to detect adulteration with very, very minute quantities of extraneous, though similar substances.

From the foregoing it becomes evident that serological techniques have an important place in food regulatory work and that they can prove to be of real value in the maintenance of the high standards of quality and purity in food products which Americans have come to know and expect.

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ANALYSIS OF LEMON OILS*

By J. W. SALE, et al.† (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

I. BY SPECTROPHOTOMETER IN THE ULTRAVIOLET RANGE

In 1929, Morton (1) reviewed the literature on absorption spectra of essential oils and perfumery chemicals, both in the visible region and in the ultraviolet, and reported data obtained in the Spectroscopic Laboratory of the University of Liverpool on a fairly large number of essential oils. He stated that lemon oil shows a band near 311 millimicrons (mμ), but that only one sample had been studied. In 1935, Brice, then Associate Physicist in the Food and Drug Administration, reported the results of an investigation of the spectral transmission of lemon oil samples (2) at wave lengths between approximately 400 and 720 mμ, and concluded in part that distilled lemon oils can be distinguished from pressed lemon oils because of their negligible chlorophyll content. The investigation was not pursued because the procedure would not serve to differentiate between cold pressed oil and distilled lemon oil that had been "refreshed" by allowing it to stand in contact with lemon peel.

Lemon oil was not included in the report (1937) by Van Os and Dykstra (3) on the examination of the ultraviolet absorption of essential oils.

In 1944 Winkler determined the absorbancy of numerous California lemon oils, including five of known purity, between the wave lengths 210 and 400 mμ. The range between 270 and 380 mμ was found to be the most useful. Similar investigations were conducted in 1945, 1951, and 1952. In 1951 the work was extended to include 12 samples of Italian lemon oil, collected by a representative of the Food and Drug Administration from factories in Sicily. During each investigation samples of distilled lemon oils, and of pressed oils from factory or warehouse stocks, were also collected and examined.

A typical absorption curve in the ultraviolet range of a representative California cold pressed oil is shown in Figure 1. This figure also illustrates how the line CD, as used herein, is obtained. Genuine cold-pressed lemon oil shows a characteristic absorbancy maximum at a wave length approximating 315 mμ. As with virtually all values obtained on substances of plant origin, this point is not entirely constant but varies to some degree according to the source. A curve for a distilled lemon oil is also shown.

Absorption data for 29 samples of California lemon oils and the 12 samples of Italian lemon oil, all of known purity, are summarized in Table 1

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† The work was planned and directed by the author of record, assisted by W. O. Winkler, who together with the following inspectors and chemists, collected and analyzed the samples: M. J. Gnagy, F. L. Hart, S. M. Hess, J. K. Kinney, G. Kirsten, J. Marder, D. C. Miller, and G. R. Wood.

together with information regarding method of manufacture. The standard deviation of absorbancy values is included. Ultraviolet absorption curves were obtained on an alcohol solution containing 0.25 gram oil in 100 ml. A Cary recording spectrophotometer was used except in the 1944 series where a Beckmann Model DU quartz spectrophotometer and dilutions of about 0.2 gram oil in 100 ml were used. The values were calculated

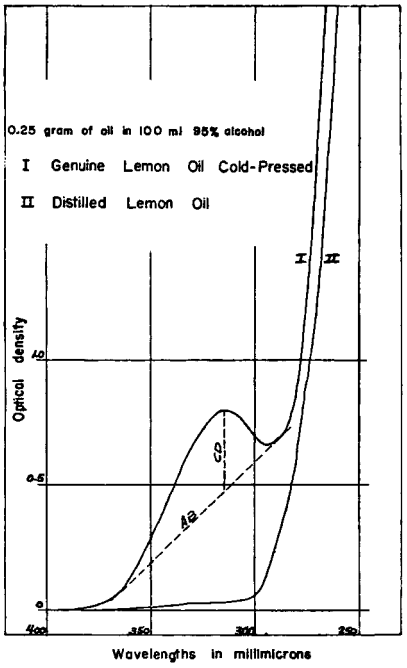

FIG. 1.—Ultraviolet Absorption Curves.

to 0.25 gram oil in 100 ml. Line CD was obtained by drawing line AB tangent to the points of inflection of the curve at approximately 285 mμ (285–295) and approximately 370 mμ (365–370) and dropping the vertical line CD from the point of peak absorption (approximately 315 mμ) to line AB.

It is a practice in California to lower the wax content of lemon oil by storage at low temperatures and subsequent filtration. To determine if dewaxing is a factor in shaping the ultraviolet curves, Mr. Winkler of the Division of Food washed a dozen lemons with benzene, removed the solvent by distillation and evaporation, took up the residue in alcohol, and measured the ultraviolet absorption of the solution (38.6 mg wax/100 ml). It showed no absorption peak at 315 mμ. In Table 1, all Subs No. 2

TABLE 1.—*Ultraviolet absorption data*

SAMPLE NO.	DATE OF COLLECTION	BASE-LINE ABSORPTION (LINE CD)	TOTAL ABSORPTION AT PEAK (CA. 315 mμ)
	California lemon oils		
INV 14-505 F Sub 3	Feb. 1944	0.36	0.77
INV 14-509 F Sub 2		0.33	0.68
INV 14-510 F Sub 2		0.23	0.53
INV 14-511 F Sub 2		0.27	0.61
INV 14-513 F	March 1944	0.48	0.96
INV 30-506 H	June 1945	0.49	1.02
INV 30-507 H		2.29	0.83
INV 30-508 H	May 1945	0.42	0.92
INV 74-339 F		0.46	0.91
INV 74-340 F		0.28	0.68
INV 86-742 K Sub 1	June 1951	0.31	0.73
INV 86-743 K Sub 1		0.34	0.76
INV 86-743 K Sub 2		0.34	0.79
INV 86-744 K Sub 1		0.32	0.68
INV 86-744 K Sub 2		0.28	0.65
INV 86-745 K Sub 1		0.39	0.80
INV 87-469 K Sub 1	May 1952	0.25	0.71
INV 87-469 K Sub 2		0.30	0.73
INV 87-470 K Sub 1		0.43	1.05
INV 87-471 K Sub 1		0.30	0.72
INV 87-471 K Sub 2		0.34	0.81
INV 87-472 K Sub 1		0.37	0.84
INV 87-473 K Sub 1		0.49	1.07
INV 87-473 K Sub 2		0.52	1.04
INV 87-474 K Sub 1		0.74	1.50
INV 87-475 K Sub 1		0.27	0.72
INV 87-475 K Sub 2		0.27	0.73
INV 87-476 K Sub 1		0.26	0.82
INV 87-476 K Sub 2		0.24	0.85
Maximum		0.74	1.50
Minimum		0.23	0.53
Average		0.36	0.82
Std. Dev.		0.11	0.19
	Italian lemon oils		
INV 14-041 K		0.79	1.44
INV 14-043 K		0.85	1.54
INV 14-044 K		0.60	1.23
INV 14-046 K		0.84	1.47
INV 14-047 K		0.76	1.40

TABLE 1—*continued*

SAMPLE NO.	DATE OF COLLECTION	BASE-LINE ABSORPTION (LINE CD)	TOTAL ABSORPTION AT PEAK (CA. 315 mμ)
	Italian lemon oils—continued		
INV 14-048 K		0.59	1.30
INV 14-049 K		0.84	1.49
INV 14-050 K		0.54	1.08
INV 14-051 K		0.77	1.60
INV 14-052 K		0.96	1.70
INV 14-053 K		0.56	1.26
INV 14-054 K		0.49	1.00
Maximum		0.96	1.70
Minimum		0.49	1.00
Average		0.71	1.38
Std. Dev.		0.15	0.21

NOTES TO ACCOMPANY TABLE 1

CALIFORNIA LEMON OILS

Sample No.	*Mfr's. No.*	*Remarks*
INV 14-505 F Sub 3	1	Centrifuged twice followed by dewaxing.
INV 14-509 F Sub 2	5	From final centrifuge fitted with a clarifying sleeve. Not dewaxed.
INV 14-510 F Sub 2	6	Centrifuged three times followed by dewaxing.
INV 14-511 F Sub 2	7	Centrifuged twice followed by dewaxing.
INV 14-513 F	9	From second centrifuge with clarifying sleeve, not dewaxed.
INV 30-506 H	9	Same as INV 14-513 F.
INV 30-507 H	9	Made from shaved off flavedo "Dark oil." From second centrifuge, not dewaxed.
INV 30-508 H	2	From second centrifuge, not dewaxed.
INV 74-339 F	1	From third centrifuge after washing with 0.5 percent citric acid solution.
INV 74-340 F	5	From second centrifuge. Same process as INV 14-509 F.
INV 86-842 K Sub 1	12	Centrifuged twice, not dewaxed.
INV 86-743 K Sub 1	1	After acid rinse, dewaxing and filtering.
INV 86-743 K Sub 2	1	From centrifuge, not dewaxed.
INV 86-744 K Sub 1	10	After dewaxing and filtering with aid of diatomaceous earth.
INV 86-744 K Sub 2	10	Same as Sub 1.
INV 86-745 K Sub 1	5	From centrifuge, not dewaxed.
INV 87-469 K Sub 1	2	From centrifuge, before storage.
INV 87-469 K Sub 2	2	From centrifuge after storage at room temperature, partly dewaxed.
INV 87-470 K Sub 1	5	From centrifuge after pressing, not dewaxed.
INV 87-471 K Sub 1	10	From centrifuge before dewaxing.
INV 87-471 K Sub 2	10	Same as Sub 1 after dewaxing and filtering.

NOTES TO TABLE 1—*continued*

CALIFORNIA LEMON OILS

Sample No.	*Mfr's. No.*	*Remarks*
INV 87-472 K Sub 1	5	Same as INV 87-470 K, Sub 1, after dewaxing.
INV 87-473 K Sub 1	11	From last centrifuge. Not dewaxed.
INV 87-473 K Sub 2	11	Same as Sub 1, after dewaxing.
INV 87-474 K Sub 1	1	From centrifuge after third centrifuging before dewaxing.
INV 87-475 K Sub 1	12	From centrifuge after second centrifuging, before dewaxing.
INV 87-475 K Sub 2	12	Same as Sub 1, after dewaxing.
INV 87-476 K Sub 1	9	From centrifuge after second centrifuging, before dewaxing.
INV 87-476 K Sub 2	9	Same as Sub 1, after dewaxing.

ITALIAN LEMON OILS

Sample No.	*Mfr's. No.*	*Remarks*
INV 14-041 K	13	By sfumatrice (4) process from lemons vicinity Palermo. Sample from separator.
INV 14-043 K	14	By sfumatrice process from lemons vicinity Bahgeria. Sample from sponges and flotation tank.
INV 14-044 K	15	Same as INV 14-043 K
INV 14-046 K	16	By sfumatrice process from lemons vicinity Carini. Sample from sponges and flotation tank.
INV 14-047 K	17	By sfumatrice process from lemons vicinity Calatabiano. Sample from separator.
INV 14-048 K	18	By sfumatrice process from lemons in area of Acireale. Sample from flotation tank.
INV 14-049 K	19	By sponge process from lemons vicinity Villafranca Tirrena. Sample from container by decantation.
INV 14-050 K	19	By sfumatrice process from lemons vicinity Villafranca Tirrena. Sample from sponges and flotation tank.
INV 14-051 K	19	By hand operated press and hollow sponge process from lemons vicinity Villafranca Tirrena. Sample by decantation.
INV 14-052 K	20	By sponge process from lemons in area of Capo d'Orlando. Sample by decantation.
INV 14-053 K	21	By sfumatrice process from lemons vicinity Capo d'Orlando. Sample from separator.
INV 14-054 K	22	By sfumatrice process from lemons vicinity Capo d'Orlando and Villafranca Tirrena. Sample from separator.

of the 1952 series of samples as well as INV 87–472 K were dewaxed, but this did not appear to change materially the shape of the curves.

Distilled lemon oil is cheaper than cold-pressed oil and is sometimes used to adulterate the latter product. Such admixture can be detected,

as evidenced by the results in Table 2 which were obtained on varying mixtures of the two types of oil.

TABLE 2.—*Detection of distilled lemon oil*

MIXTURE	BASE-LINE ABSORPTION (LINE CD)	TOTAL ABSORPTION AT PEAK (CA 315 mμ)
100% cold-pressed lemon oil	0.34	0.79
15% w/w distilled lemon oil	0.26	0.64
30% w/w distilled lemon oil	0.20	0.54
60% w/w distilled lemon oil	0.09	0.32
95% w/w distilled lemon oil	0.01	0.07

In a number of instances products labeled "Lemon Oil" and "U.S.P. Lemon Oil" showed ultraviolet absorption values which deviated so widely from those of the authentic samples that they were classed as adulterated even though the usual constants: refractive index, optical rotation, specific gravity, citral, esters, etc., were within the ranges of those for genuine lemon oil. However, as will appear from the second part of this paper, in deciding whether a suspect sample is adulterated, not only the ultraviolet curve, but also the usual chemical and physical criteria of purity should be considered.

At present ultraviolet absorption values of fractions of lemon oils of known purity, obtained by distillation and by the chromatographic column are being investigated.

II. BY CHEMICAL MEANS

In the analysis of lemon oil, evaporation residue is frequently determined even though many California lemon oils are dewaxed by storing at low temperatures and filtering. This process of course reduces the amount of residue.

The residue obtained by ordinary evaporation appears to be subject to uncontrollable variations due to oxidation of the unsaturated compounds on prolonged boiling. The writers prefer the Winkler steam distillation residue procedure rather than the ordinary evaporation method. A modified Seeker and Kirby method (5) for esters in lemon oil and a slightly modified British Pharmacopoeia method for total aldehydes expressed as citral are also employed. These procedures are described by Winkler (6) in the following paper.

Results obtained by these three methods, together with aldehyde-ester ratios on all of the samples except the 1952 series, are contained in Table 3 and require no particular comment. Optical rotation and refractive indices of the original Italian oils and of their 10 per cent distillates (7) were determined, with the exception of the 10 per cent distillate of INV 14-051 K, and found to be within the ranges specified in U. S. Pharma-

TABLE 3.—*Chemical analysis of lemon oils of known purity*

	STEAM DISTILLATION RESIDUE	TOTAL ALDEHYDES AS CITRAL†	ESTERS AS LINALYL ACETATE	RATIO OF TOTAL ALDEHYDES AS CITRAL TO ESTERS
	per cent	*per cent*	*per cent*	
		California Lemon Oils		
INV 14-505 F Sub 3*	2.04	2.94	2.27	1.30
INV 14-509 F Sub 2	1.57	2.67	2.27	1.18
INV 14-510 F Sub 2*	1.52	3.09	1.97	1.57
INV 14-511 F Sub 2*	2.91	2.34	1.95	1.20
INV 14-513 F	4.01	3.14	3.43	0.92
INV 30-506 H	3.05	2.01	3.04	0.66
INV 30-507 H	5.05	2.16	2.36	0.92
INV 30-508 H	2.71	1.76	2.36	0.75
INV 74-339 F	3.14	2.75	2.94	0.94
INV 74-340 F	2.41	2.46	2.32	1.06
INV 86-742 K Sub 1	2.01	2.52	2.78	0.91
INV 86-743 K Sub 1*	1.97	2.59	2.09	1.24
INV 86-743 K Sub 2	2.62	2.69	1.92	1.40
INV 86-744 K Sub 1*	2.39	2.0	1.73	1.16
INV 86-744 K Sub 2*	2.95	2.20	2.02	1.09
INV 86-745 K Sub 1	2.49	1.78	1.98	0.90
Average (16)	2.25* 3.01	2.44	2.34	1.07
		Italian Lemon Oils		
INV 14-041 K	2.01	3.86	2.07	1.86
INV 14-043 K	1.83	3.73	2.13	1.75
INV 14-044 K	1.78	3.49	1.92	1.82
INV 14-046 K	2.25	3.73	2.24	1.66
INV 14-047 K	2.05	3.98	2.10	1.89
INV 14-048 K	1.56	4.03	1.70	2.37
INV 14-049 K	1.80	4.37	2.03	2.15
INV 14-050 K	1.50	3.77	1.57	2.40
INV 14-051 K	2.18	4.22	2.04	2.07
INV 14-052 K	2.02	4.32	2.19	1.97
INV 14-053 K	1.78	3.97	1.67	2.38
INV 14-054 K	1.70	4.08	1.56	2.62
Average (12)	1.87	3.96	1.93	2.08

* Dewaxed.
† INV 14-505/11 F and 14-513 F by Brit. Pharm. method, others by Brit. Pharm. method as modified by Winkler.

copoeia XIV. The amount of INV 14-051 K was insufficient to allow completion of the analysis.

ADDENDUM

After submission of this paper, Mr. Gordon Wood called the writers' attention to an article in a new Italian publication (8) containing analyses of Sicilian lemon

oils and including absorption data. The article was obtained through the courtesy of Dr. Aldo Buffa of Palermo. Dr. Buffa and his associates used alcohol solutions containing 0.05 per cent lemon oil and reported their findings as per cent transmission at wave lengths of 270, 280, 290, 300, 310, 320, 330, and 340 mμ. Their results are in excellent agreement with ours, as will be observed from the following summary of their absorption data at 310 mμ, calculated to absorbancy of 0.25 gram lemon oil in 100 ml alcohol.

ITALIAN LEMON OILS

	Absorption at 310 mμ
Maximum	1.63
Minimum	1.02
Average (34 samples)	1.34
Standard deviation	0.23

The authors state that the samples were collected during their industrial production, but do not further indicate specific dates of collection. They do state that by tracing a line that connects the highest transmission points, and a second that connects the lowest values (always in relation to pure essential oils), one obtains an area which should comprise all the transmission values characteristic of genuine essential lemon oils examined under the described conditions. This is in accord with our own experience with respect to Italian lemon oils.

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METHODS FOR THE EXAMINATION OF LEMON OIL*

By W. O. WINKLER, (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

Methods for analyzing citrus oils have followed a fixed pattern with very little change for many years and chemists seem slow in the application of tools found valuable in other fields. As mentioned in the preceding paper (1) the writer in 1944 began using the spectrophotometer in the examination of lemon oil and was at the time unaware that such examination had been previously reported. The instrument has proved to be an

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excellent tool for the detection of foreign substances or of dilution with distilled oil or terpenes. Absorption curves and data for lemon oils of known purity are given in the above publication and are found to follow a definite pattern.

At that time (1944) the writer also developed a method for the determination of evaporation residue (total solids) by steam distillation which has pronounced advantages over the usual method. In addition, the writer modified the Seeker and Kirby (2) method for esters, and later the British Pharmacopoeia (3) method for aldehydes. These new or modified methods are described here.

RESIDUE BY STEAM DISTILLATION

APPARATUS

The apparatus is similar to that used for volatile acids; *Methods of Analysis, A.O.A.C.*, 7th Ed., page 298, except that a 250 ml distillation flask is used.

DETERMINATION

Add 50 ml of water and 15 ml of sample to the 250 ml distillation flask. Weigh 15 ml of the oil delivered by the same pipet to obtain weight of sample. Place a steam inlet tube (same as used for volatile acids) in the flask, heat the contents of flask just to boiling, and connect inlet tube to steam source. Adjust the flame so that the water level remains about the same. Steam distill at rate of about 200 ml per hour until 100 ml of water are collected. Discontinue distillation and allow flask to partially cool, then decant contents of flask into a separatory funnel (125–250 ml) and allow it to drain. Rinse the flask twice with 15 ml and 8 ml portions of alcohol, warming if necessary to dissolve any residue. Pour the alcohol rinsings into a tared 150 ml beaker. Extract the cooled liquid in the separatory funnel twice with $CHCl_3$ using 25 and 20 ml portions respectively. (Add a drop or two of HCl (1+2) to the funnel if there is any tendency for the liquids to emulsify.) Add the extracts to the tared beaker containing the alcohol washings; then extract once with 25 ml of ethyl ether and unite this extract with the others. Evaporate the extracts carefully without spattering on cover of steam bath until the ether and chloroform are removed. Then evaporate the residual liquid on the open steam bath. Allow the beaker to remain on the bath for 15 minutes after odor of solvent (alcohol) disappears. Remove and wipe the outside of beaker with clean dry cloth, allow to cool, and weigh. Calculate per cent residue by steam distillation.

A few typical results on authentic Italian and Sicilian oils are given in Table 1.

As observed in the table, there is a marked difference between the residues on the fresh whole oil and the distilled oil. If the sample has been allowed to deteriorate the difference is not so marked. The residues obtained by direct evaporation of distilled oils are often fairly high due to oxidation and other changes during the heating.

MODIFIED SEEKER & KIRBY METHOD FOR ESTERS

The ester method of Seeker and Kirby (2) was modified to increase the ease of operation, reduce the tendency to form emulsions, and to obtain a sharper end point.

TABLE 1.—*Results obtained from Italian and Sicilian oils by steam distillation*

SAMPLE	PROCESS	RESIDUE
		per cent
1	Sfumatrice flotation separation	1.56
2	Hand pressed sponge process	2.06
3	Hand lever press oil	2.18
4	Hand pressed sponge process	2.02
5	Sfumatrice separator	1.78
6	Distilled oil	0.13
7	Distilled oil	0.04

DETERMINATION

Weigh 5 ml of oil in a beaker or bottle and transfer to a 125 ml separatory funnel, using exactly 25 ml of alcohol to wash the weighing vessel and complete the transfer. Add 1 ml of 50% hydroxylamine hydrochloride, some phenophthalein indicator, and mix. Add from buret or graduated pipet sufficient 4% KOH in 80% alcohol to make the solution just pink to the indicator and add a drop or so in excess. Add 1 drop of 20% hydroxylamine hydrochloride and shake; the pink color should be discharged.

Introduce 25 ml of isoamyl alcohol (aldehyde free)* and shake. Then add 50 ml NaCl solution (160 grams dissolved in 500 ml of water), shake vigorously, allow layers to separate (line of division should be sharp) and draw off and discard the lower layer. Repeat the extraction with 3 successive 25 ml portions of the salt solution and once with 6 ml of water, drawing off and discarding extracts each time. Run the remaining amyl alcohol-oil layer into a 500 ml Erlenmeyer flask. Wash the funnel once with 25 ml of ethyl alcohol and combine the wash alcohol with the solution in the flask. Add phenolphthalein indicator and make the liquid just pink with approximately 0.2 *N* standard KOH and then add from a pipet exactly 20 ml of the standard alkali in excess.

Reflux the solution for 45 minutes on a hot plate, then cool with the flask loosely stoppered. Add about 200 ml of CO_2 free H_2O and 8 drops of 1% phenolphthalein indicator. Titrate the excess KOH with 0.2 *N* HCl (or other standard) until the pink color is almost gone. Complete the titration as follows:

When the end point is approached, add the 0.2 *N* acid to the Erlenmeyer in 0.5 ml portions until the color disappears (even when additional indicator is added) and an excess of about 0.5 ml has been added. Then transfer the liquid in the flask to a 500 ml separatory funnel. Allow layers to separate and draw off the lower aqueous layer into a 600 ml beaker. Wash the flask and the liquid remaining in the funnel with two 70 ml portions of water. Add indicator to the beaker and back titrate with the standard KOH solution until the liquid is pink again, then complete the titration to the disappearance of the pink color with the 0.2 *N* standard acid.

Conduct a blank determination in the same manner as the sample, using the same amounts of all reagents. Subtract the titration of the sample from that of the blank to obtain the equivalent of 0.2 *N* alkali consumed. Calculate esters from the relation: 1 ml of 0.2 *N* alkali = 39.2 mg. of esters as linalyl acetate.

* Reflux about 1 liter of reagent grade isoamyl alcohol over 35–40 grams of KOH for 60–70 minutes. Distill in all glass apparatus and reject first 25 ml of distillate. Use succeeding distillate leaving ca 125 ml of liquid in distillation flask. Preserve in cold at about 40°F.

A few results by the above method on authentic California cold pressed oils, as compared with the direct hydrolysis method of Romeo and Guiffre (4); are given in Table 2. It is seen that results by the modified method are lower than by the method of Romeo and Guiffre. This is to be expected since the latter would be more affected by interferences such as aldehydes or lactones.

TABLE 2.—*Esters found in California lemon oils*

SAMPLE NUMBER	R. AND G. METHOD	MODIFIED S. AND K. METHOD
	per cent	*per cent*
14-508	2.59	2.25
14-509	2.78	2.27
14-510	2.26	1.97
14-511	2.08	1.95
14-513	3.90	3.43

DETERMINATION OF TOTAL ALDEHYDES IN LEMON OIL BY HYDROXYLAMINE METHOD

A weakness of most methods for aldehydes or citral in lemon oil is their inability to yield sharp end points, and the phenylhydrazine method seems particularly bad in this regard. The British Pharmacopoeia method which employs hydroxylamine hydrochloride (a general aldehyde reagent) is less subject to this objection. The following modified method, devised by J. B. Wilson, of the Division of Food, and the writer, gives a readily discernible end point, and duplicate determinations can be checked to .05 ml of the hydroxylamine reagent.

REAGENTS

(a) *Bromophenol blue.*—Dissolve 0.1 g bromophenol blue in 5 ml of 0.05 *N* NaOH and dilute to 100 ml with 60 per cent alcohol.

(b) *Ethyl orange.*—Dissolve 0.05 g of ethyl orange in 60 per cent alcohol and make up to 50 ml.

(c) 0.5 *Potassium hydroxide reagent.*—Dissolve 28.05 g KOH in 60 per cent alcohol and dilute to 1 liter with the same solvent. Standardize, using standard HCl.

(d) *Hydroxylamine reagent.*—Dissolve 7.0 g of hydroxylamine hydrochloride in 175 ml of alcohol (60 per cent). To that solution add: (1) 0.3 ml bromophenol blue indicator and sufficient 0.5 *N* potassium hydroxide reagent to give a permanent blue color, or (2) 0.3 ml ethyl orange indicator and sufficient 0.5 *N* potassium hydroxide reagent to give a permanent yellow color. In either case dilute the resulting solution to 200 ml with the 60 per cent alcohol.

DETERMINATION

Weigh to the nearest centigram about 10 g of the oil into a glass-stoppered 50 ml graduate and add 7 ml of hydroxylamine reagent and 0.1 ml of indicator. Shake, and neutralize the liberated acid with the 0.5 *N* KOH reagent to the permanent full alkaline color of the indicator used. Continue shaking and neutralizing until a permanent alkaline color remains in the lower layer after shaking the mixture

vigorously for 2 minutes and allowing separation to take place. (The reaction is complete in about 15 minutes.) Each ml of 0.5 *N* KOH is equivalent to 0.0761 g of citral.

This procedure gives an approximate determination of citral in the oil. Carry out a second determination in exactly the same manner using as the color standard for the end-point the titrated liquid of the first determination. The volume of the hydroxylamine hydrochloride reagent should be varied according to the citral content of the oil and should exceed by 1 to 2 ml the volume of the 0.5 *N* potassium hydroxide reagent required.

The results reported in the previous paper (1), were obtained by this method.

It has been known for a good many years that citrus oils deteriorate on exposure to air and light. This fact may not be generally realized by manufacturers and chemists. To best preserve the oil three conditions are necessary: (1) the container should transmit little or no light or the oil should be kept in a dark place, (2) the container should be kept full or practically so (or the space above the oil should be filled with an inert gas), (3) the oil should be stored in a cool place. The author makes it a practice to divide the sample into small portions of 1, 2, or 4 fluid ounces and to store them in well filled amber bottles at 40°F. The bottles are glass stoppered, or screw capped with foil lining. Under these conditions lemon oil may be kept for a considerable time and still retain its flavor, freshness, and ability to yield a reproducible analysis.

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THE CONTENT OF URONIC ACIDS IN SEVERAL GRADES OF FLUE-CURED, TYPE 12, TOBACCO*

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The uronic acids are aldehydic monocarboxylic sugar acids and are widely distributed in the vegetable kingdom. They are the principal building units of such polyuronides or polymerized uronic anhydrides as the pectic substances. They occur also in most hemicelluloses, gums, and in mucilages in conjugation with hexoses and pentoses.

* The investigation on which this report is based was conducted with funds made available under the authority of the Agricultural Marketing Act of 1946 (RMA, Title II).

Although a large number of uronic acids are theoretically possible, only three aldohexuronic acids have thus far been found to occur in nature. d-Galacturonic acid is the uronic acid generally present in the pectic substances (1), while in the hemicelluloses both d-glucuronic acid and d-galacturonic acid have been shown to occur (2). d-Glucuronic acid and d-galacturonic acid are the only uronic acids that have thus far been found to occur in seed plants, though d-mannuronic acid has been shown to be present in certain algae (3).

When a uronic acid is boiled with 12 per cent hydrochloric acid, it is decomposed into water, furfural, and carbon dioxide. The yield of furfural is less than theoretical, while the amount of carbon dioxide liberated is quantitative, and for one mole of uronic acid, one mole of carbon dioxide is produced. This is the basis of the method first developed by Lefèvre and Tollens (4) for the quantitative estimation of uronic acids. This method has since been modified and improved in certain respects by several investigators (5). The results are generally expressed as per cent of uronic acid anhydride. Since the molecular weight of the anhydride of an aldohexuronic acid is 176 and that of carbon dioxide 44, it follows that 4 times the percentage of carbon dioxide found is the percentage of uronic acid anhydride in the sample.

Although the literature on the chemistry of tobacco is now rather extensive, and numerous organic constituents, groups, or fractions have been determined, no information is available on the content of uronic acids in tobacco. This is rather surprising, inasmuch as there are fairly accurate and specific methods available for the determination of these acids. The work described in this paper, which is of a preliminary nature, was undertaken for the purpose of determining the uronic acids content of various grades of Type 12, American flue-cured tobacco, and also to ascertain whether there was any relationship between the percentages of uronic acids and the various physical characteristics and quality factors of the several grades examined.

EXPERIMENTAL

Selection and preparation of the several grades of tobacco.—All tobacco grades examined were of Type 12 of the 1949 crop. The tobacco samples were selected from the most uniform farm-sorted lots appearing on the Wilson and Kinston, North Carolina, auction markets. The samples were shipped to Washington where they were carefully re-sorted to reduce each to a high degree of uniformity. These samples were so sorted as to represent the basic Federal grades, without allowance for the different degrees of mixtures and irregularities commonly found in farm sorted tobacco. Sample 91, designated as "waste," consisted of field-burnt and badly decomposed tobacco and was of no commercial value. It was included here for special study and for comparative purposes only.

In all cases, the stems or midribs were completely removed and the residual leaf tissue was dried for four days at ordinary room temperatures. It was then granulated by hand until the tobacco passed through a sieve having 4 mm square openings. The tobacco was then sifted in a sieve having 0.5 mm square openings, and the material

which passed through was discarded. The tobacco remaining in the sieve was ground in a Wiley mill provided with a 1 mm sieve, thoroughly mixed, and preserved in jars provided with air-tight closures.

In order to eliminate any error resulting from the evolution of small quantities of carbon dioxide when certain sugars are boiled with 12 per cent hydrochloric acid (5e), all samples were first extracted for 16 hours in a Soxhlet extractor with 80 per cent ethanol, and loss in weight due to this extraction was determined. The uronic acid determinations were made on the dried extracted tobacco and the results were calculated on the original moisture- and sand-free, stemmed tobacco basis. Moisture in the original stemmed, air dry and ground tobacco samples was determined by drying for 4 hours at 100°C. and calculating the loss in weight as moisture. The percentage of sand was determined following the procedure described in *Methods of Analysis*, A.O.A.C., 7th Ed., p. 94.

DETERMINATION OF URONIC ACIDS

APPARATUS

The apparatus described by Browning (5i) was used. However, anhydrous calcium chloride was used in place of anhydrone in the long drying tube, absorption tube, and guard tubes. The anhydrous calcium chloride was pretreated as follows: It was placed in a tube of suitable size and a slow stream of dry carbon dioxide was passed through for one-half hour. A stream of dry air, free of carbon dioxide, was then passed through for one hour. The anhydrous calcium chloride was then preserved in a well stoppered bottle and used as needed.

PROCEDURE

The analytical method used was a modification of that of Browning (5i). One gram of the 80 per cent ethanol extracted tobacco, 60 ml of 12 per cent hydrochloric acid and two boiling chips ("Boileezers") were added to the reaction flask. It was connected to the apparatus, and the glycerol bath was heated at such a rate that the temperature was raised to 70°C. in 20 minutes. During this time, as well as throughout the determination, dry air, freed of carbon dioxide, was passed through the apparatus at the rate of two to three bubbles per second. The purpose of this preliminary treatment was to decompose carbonates, as recommended by Phillips, Goss, and Browne (6), and also to flush out carbon dioxide from the apparatus. The absorption tube was then disconnected, conditioned in a stoppered test tube near the balance for five minutes, and weighed. During this time, the temperature of the glycerine bath containing the reaction flask was maintained at 70°C. and the air, freed of carbon dioxide, was allowed to pass through the apparatus at the rate of two to three bubbles per second. The absorption tube was again connected to the apparatus, and the temperature of the glycerol bath was raised to 137–140°C. in the course of about 30 minutes, and kept at this temperature for five hours. At the end of the five-hour period, the absorption tube was disconnected, conditioned in a stoppered test tube near the balance for five minutes, and weighed. The gain in weight, corrected for the carbon dioxide found in a blank determination, represented the carbon dioxide given off by the uronic acids. The percentage of carbon dioxide multiplied by 4 gave the percentage of uronic acid anhydrides in the sample.

The results (Table 1) were calculated on the basis of the original unextracted tobacco, both on a moisture-free and moisture- and sand-free basis. The several grades are arranged in the table into three blocks. This division is based primarily on three standardization factors, namely, degree of body, relative tensile strength, and per cent of waste. Block I consists of seven grades which are relatively heavy in body, and have the

TABLE 1.—*Percentages of uronic acids (as anhydrides) in certain grades of type 12 tobacco*†

	SAMPLE NO.	U. S. GRADE	URONIC ACIDS (AS ANHYD.) ($CO_2 \times 4$)	
			MOISTURE-FREE BASIS	MOISTURE-FREE & SAND-FREE BASIS
			per cent	*per cent*
	78	B2F	9.44	9.50
	79	B3LV	10.68	10.70
	92	B4GF	10.80	10.81
I	94	B5L	10.44	10.52
	81	H2L	10.52	10.58
	80	H3F	9.84	9.89
	82	C2F	9.28	9.37
	Av.		10.14	10.20
	95	H5L	11.76	11.81
	97	C5L	11.72	11.86
	96	C5F	11.04	11.17
	84	X3L	10.52	10.71
II	98	X3F	10.88	11.08
	83	X3F	10.96	11.23
	86	P3L	11.60	11.89
	99	P3F	10.60	11.10
	85	P3F	10.88	11.32
	Av.		11.11	11.35
	87	P4L	11.44	11.73
	88	P4F	12.00	12.37
	100	X5L	12.32	12.62
III	101	X5F	12.92	13.22
	89	P5F	12.24	12.64
	90	N2L	12.44	12.85
	91	Waste	13.84	14.53
	Av.		12.46	12.85

† Analyses were made only on stemmed tobacco. Results are the averages of at least two determinations concordant within the limits of experimental error.

greatest tensile strength and the least injury and waste. The six grades of Block III are distinctly thinner in body, and have less tensile strength and substantially more waste than those of Block I. The grades of Block II are intermediate between those two extremes with respect to the three aforesaid standardization factors.

There is a fairly close relationship between the grades in each block, except in the case of Block III, which includes a sample (No. 91) made up

of 100 per cent of waste. The six grades in Block III are known in the tobacco trade as "granulators" owing to the fact that they are thin tobaccos which do not have sufficient tensile strength to hold together well when they are finely cut for cigarette purposes.

Table 1 shows that the uronic acids occur in stemmed flue-cured tobacco in fairly substantial quantities. They do not, of course, occur in the free state, but serve as building units for, and form an integral part of such polyuronides as the pectic substances, hemicelluloses, gums, and perhaps of other tobacco constituents.

The lowest and highest average percentages of uronic acids (as anhydrides) were found in Blocks I and III respectively. The average percentage of uronic acids (as anhydrides) of Block II were found to be intermediate between those of Blocks I and III.

There is a relationship between the percentage of uronic acids and the content of "waste" as defined in the flue-cured tobacco standards (7). The term "waste" is used to designate tobacco which has been affected by field diseases, injured by drought or other causes, and has undergone decomposition. Block I, which contained the lowest percentage of waste, had the lowest percentage of uronic acids, while Block III had the highest percentage of waste and also the highest percentage of uronic acids. It will be noted that sample 91, which consisted entirely of tobacco designated as "waste," contained the greatest percentage of uronic acids. Presumably the polyuronides are relatively more resistant than some of the other tobacco constituents to that type of microbial or other decomposition which produces tobacco waste, with the result that the residual tobacco contains a greater percentage of uronic acids.

There also appears to be a direct relationship between the content of uronic acids (as anhydrides) and what is called mildness in tobacco. Thus, the milder tobaccos of Block III contained greater percentages of uronic acids than those of Blocks I and II.

In Block II of the table are included duplicate samples of grades X3F and P3F (Nos. 98, 83, and 99, 85 respectively). The tobacco samples in each case were collected and re-sorted independently. It will be noted that in both cases reasonably close agreements were obtained with respect to the percentages of uronic acid anhydrides.

The percentage of uronic acids was also determined in one sample which was made up of leaves selected from different farm lots to represent a very unripe or crude green tobacco. This sample was graded N2G and the percentage of uronic acid anhydrides in this tobacco, calculated on a moisture and sand-free basis, was 11.19.

SUMMARY

Data are presented on the content of uronic acids (as anhydrides) in 23 grades of stemmed, flue-cured, Type 12, tobacco and of one sample of

tobacco "waste." The results show that, in general, the tobacco grades which were relatively heavier in body and had the greatest tensile strength contained the lowest percentage of uronic acids as anhydrides, while those which were distinctly thinner in body and had the least tensile strength had the highest percentage of uronic acid anhydrides. The percentages of uronic acids (as anhydrides) were also found to be directly related to the content of "waste" and possibly also mildness in tobacco.

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PREPARATION OF SAMPLE OF PRESSURIZED CREAM*

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Whipped cream type toppings, sold in dispensing, pressurized containers are finding increasingly widespread use both in the home and in restaurants, and many of these products have appeared on the market. While there is no standard of identity for this type product, the need exists for suitable methods of analysis.

* Presented at the 66th Annual Meeting of the Association of Official Agricultural Chemists, held in Washington, September 29, 30, and October 1, 1952.

The use of pressurized containers has caused certain analytical difficulties and the object of this study was to develop a satisfactory procedure for the handling and preparation of samples. Commercial cans of whipped toppings were used in these experiments, and reproducibility of results of fat analyses was considered indicative of satisfactory sample preparation.

HANDLING OF CONTAINERS

Since the simplest procedure would be to dispense the well shaken product as directed on the can, this was first tried. Approximately 2 gram charges were dispensed directly into beakers for fat analysis. It was impossible to get check determinations as the following table shows:

Fill of Container	*Per cent Fat*
Almost full	25.68
About ½ full	24.45
About ¼ full	22.74
Almost empty	21.87

In each case the fluffed material was normal in appearance whether taken from first, middle, or end portions of can. The apparently empty can was found to contain approximately 1 oz. of product with a fat content of 13.27 per cent. These results demonstrated the necessity of complete removal of the contents of the can and thorough mixing.

Dispensing the material into a Mason jar or Waring blendor, either directly or after releasing most of the pressure was usually accompanied by spattering. Furthermore, the containers eventually had to be opened to remove the residue not obtained through the nozzle.

It was finally found that, by placing the containers in the deep freezer until contents were frozen, the gas could be released without loss of sample. The cans were then opened either by cutting out the bottom or, in the case of the heavy, returnable type, by unscrewing one end with a wrench. In this frozen state, the wax lining used by some manufacturers can easily be separated from the cream mixture. The contents were then transferred directly into the cup of a Waring blendor and allowed to thaw.

PREPARATION OF SAMPLE

The initial attempts to obtain a homogeneous mixture by means of the Waring blendor proved unsatisfactory since the whipping caused a separation of the fat as butter. At this point, the samples were considered lost. It was finally discovered that prolonged, intermittent beating eventually broke down the churned fat mass and a creamy liquid was obtained. Five different brands of this type product were tried in the blendor, and, while some required longer periods of beating, all were finally whipped into a creamy, homogeneous mixture. It was easily possible to withdraw samples for analysis and, by occasionally turning on the blendor for a few

seconds, to maintain the sample in a homogeneous state. Emulsifiers, such as egg albumen or gelatin were not necessary.

A check of the homogeneous nature of the five samples prepared in this manner was made by determining the fat content by a modified rapid Babcock method (1). The results on duplicate portions were:

Brand	*Per cent Fat*	
A	22.9	22.8
B	35.9	35.9
C	23.2	23.3
D	26.2	26.4
E	23.5	23.6

METHODS OF ANALYSIS FOR FAT

There is no official method for determining fat in this type of product and various methods were tried. The modified Babcock test (1) was used because it was rapid, but the ice cream method (2) was thought to be more suitable since the toppings more nearly resemble an ice cream mix in composition. A comparison of results obtained on additional samples of topping using both the Babcock and ice cream method is given in Table 1:

TABLE 1.—*Fat by Babcock and ice cream official method*

SAMPLE	MODIFIED BABCOCK METHOD		ICE CREAM METHOD	
	per cent fat		*per cent fat*	
A	23.6	23.6	22.54	22.18
B	23.6	23.3	23.03	22.78
C	24.3	24.5	23.27	—
D	23.6	23.6	22.27	21.95

It will be observed that the Babcock method gave results which were consistently higher, and it should be noted that a previous referee (5) found that none of the numerous modifications of the Babcock test were satisfactory for the determination of fat in ice cream. In a later experiment, the cream method (3) was also tried. The results (Table 2) compared favorably with the ice cream method.

As a final check on sample preparation, two different brands of topping were prepared for analysis by freezing and by mixing in the Waring blendor. The fat by the ice cream method, also the total solids were determined in quintuplicate and are reported in Table 2. Quadruplicate results by the cream method are included for comparison.

These results indicate that a homogeneous mixture can be obtained with the Waring blendor. Samples for sucrose and other analyses may be taken as needed.

The following method is proposed for handling and preparing samples of whipped toppings.

TABLE 2.—*Solids and fat in pressurized cream, replicates*

	PER CENT SOLIDS 15.151 (4)	PER CENT FAT 15.153 (2)	PER CENT FAT 15.61 (3)
Sample I	42.89	27.35	27.46
	42.99	27.31	27.61
	42.98	27.29	27.60
	43.06	27.44	27.60
	42.96	27.56	
	Av. (5) 42.98	Av. (5) 27.39	Av. (4) 27.57
Sample II	35.12	22.48	22.46
	35.20	22.34	22.35
	35.02	22.21	22.31
	35.20	22.19	22.19
	35.20	22.32	
	Av. (5) 35.15	Av. (5) 22.31	Av. (4) 22.32

METHOD OF SAMPLING

Place containers in deep freezer (ice cream cabinet or equivalent) overnight to freeze contents. When frozen, release as much gas as possible through the nozzle, holding container upright. Open container, using a can opener on non-returnable type or a wrench on the heavier, returnable type. Empty contents into 1 l jar of the Waring blendor and allow to thaw; complete thawing is not necessary. Beat until a smooth creamy liquid is obtained, keeping the blendor covered. This step is best done by intermittent beating to prevent overheating of the sample and blendor. (This process is somewhat long and requires about 15 minutes in all.) The "butter" stage is intermediate and it is important to continue the beating until this stage is passed. Weigh out samples for fat, solids, sucrose, or other analyses, beating a few seconds between withdrawal of samples.

SUMMARY

A method is presented for the preparation for analysis of samples of whipped toppings in pressurized containers. The method involves freezing the contents of the container, releasing the gas while contents are frozen, transferring the frozen contents to a Waring blendor, and mixing thoroughly. Replicate determinations of fat and total solids indicate that a uniform sample is obtained.

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THE DETERMINATION OF MOISTURE IN PROCESS AMERICAN CHEESE AND PROCESS CHEESE FOOD*

J. H. Cook, H. C. Follstad, and W. W. Fisher (Research Laboratories, Kraft Foods Company, Glenview, Illinois)

The official method for the determination of moisture in process American cheese, process cheese food and other cheese products uses the 100°C. vacuum oven. Constant weight is obtained in the case of process American cheese in approximately four hours, but in the case of process cheese food the sample will continue to lose weight upon continued heating after the moisture has been removed. In many cases the residues take on a dark brown color and the odor of caramel is present.

It has been observed that if the sample of process cheese food is spread thinly over the bottom of the moisture dish the residue will be much lighter in color and there will be no burned or caramel odor. In addition, the moisture value obtained will be lower than that of a similar sample which is merely cut into small pieces when weighed, but is not spread out. This observation has been investigated for some time in the Kraft Laboratories and a technique worked out to utilize it. Small "mashers" were made by flattening out the ends of glass rods approximately one and one-half inches in length; these mashers were then tared with the moisture dish. A single lump of sample is quickly taken and weighed into the dish. After the weighing is complete, the masher is used to smear and spread the sample over the bottom of the dish in a thin layer and is left in the dish during the drying period.

The question arose as to whether the official method for cheese food was giving a result that was too high, or the glass rod technique was unable to remove all the moisture actually present. To solve this problem, a study was made on 108 samples of process cheese food and 105 samples of process American cheese. Fundamentally the solution could be reached in one of two ways: (1) by proving that nothing other than water is driven off by the official method, or (2) by proving that no moisture remains in the residue after the sample is dried by the glass rod technique. The first of these two obviously would be very difficult; the second would appear to be the more practical. If it can be shown that no moisture remains in a dried residue, we have obtained the "moisture value" by definition.

The original plan was to dry a number of samples by both the glass rod method and the official method and examine the residues by the Karl Fisher method to determine whether any traces of moisture remained. However, it was found that the residues strongly resisted extraction by the Fischer reagent and this approach had to be abandoned. Since the samples dried by the glass rod method were nearly always lower in mois-

* Presented at the 66th Annual Meeting of the Association of Official Agricultural Chemists, held in Washington, September 29, 30, and October 1, 1952.

ture content than the corresponding sample dried by the official method, it was suggested that the residues of the glass rod tests be returned to the oven for an additional period to see whether any further weight loss would occur. This procedure is open to the criticism that process cheese food is subject to burning, and if a weight loss does occur it might be due to something other than evolved water. On the other hand, if no further weight loss occurs, the problem would appear to be solved. Constant weight is usually assumed when no weight loss occurs after an additional hour of reheating. After some preliminary tests on the ability of the residues to withstand heat, it was decided to reheat for an additional three hours. This is drastic treatment for a product like cheese food, but should serve to remove all doubt as to whether any moisture remains in the sample.

The tests were conducted as follows; two sets of duplicates of each sample of cheese or cheese food were weighed out at the same time. One set was treated as prescribed by the official method.* The other set of duplicates was weighed up with the "masher" that was used to smear the sample in a thin layer over the bottom of the dish. Both sets were placed in the vacuum oven for five hours at 100°C. and a vacuum of 29 inches of mercury. Six maximum reading thermometers were distributed over the oven in order to be certain that no freak hot or cold spots had developed during the drying. At the end of five hours, the samples were cooled in a desiccator and weighed. The glass rod residues were then returned to the same oven for an additional three hours at 100°C. and 29 inches of vacuum.

The sampling technique was the same in all cases; namely, a block of packaged cheese or cheese food was cut in half and the sample taken from the exact center of the block. Previous studies as shown in Table 1 indicated that such a block is sufficiently homogeneous to allow sampling in this way. In the glass rod method an effort was made to secure a sample of approximately two grams in a single piece and to transfer it to the dish and weigh it quickly.

Process Cheese Food.—Upon reheating the glass rod residues of the 108 samples of process cheese food for an additional three hours, the loss in weight averaged only 0.09 per cent. Sixty-four per cent of the samples showed less than 0.1 per cent change and 93 per cent showed less than 0.2 per cent change. This would indicate that the glass rod technique and five hour drying removes all the moisture.

On comparing the glass rod results at the end of five hours with those by the official method, it is noted that the latter gives a higher result in practically every case (Fig. 1). The average difference is 0.3 per cent, but nearly 20 per cent of the samples showed a difference of 0.5 per cent and

* *Methods of Analysis, A.O.A.C.* Seventh ed., p. 262, omitting predrying on steam bath.

TABLE 1.—*Uniformity of the moisture content in products studied*

TYPE OF CHEESE ANALYZED	METHOD USED	NUMBER OF DETERMINATIONS	AVERAGE MOISTURE PER CENT	RANGE PER CENT	MOISTURE STANDARD DEVIATION
2 lb. Block, process cheese food	Glass	20	43.7	43.56 to 43.81	.042
2 lb. Block, process cheese food	Glass Rod	20	43.1	42.84 to 43.24	.093
2 lb. Block, process cheese food	AOAC	20	43.8	43.55 to 43.89	.28
2 lb. Block, process cheese food	AOAC	20	43.2	42.86 to 43.36	.143
5 lb. Block, process American	Glass Rod	20	39.9	39.76 to 40.06	.065
5 lb. Block, process American	AOAC Rod	20	39.9	39.65 to 40.91	.290
5 lb. Block, process American	Glass Rod	24	39.7	39.30 to 40.30	.18
5 lb. Block, process American	Glass Rod	24	39.7	39.40 to 40.00	.14

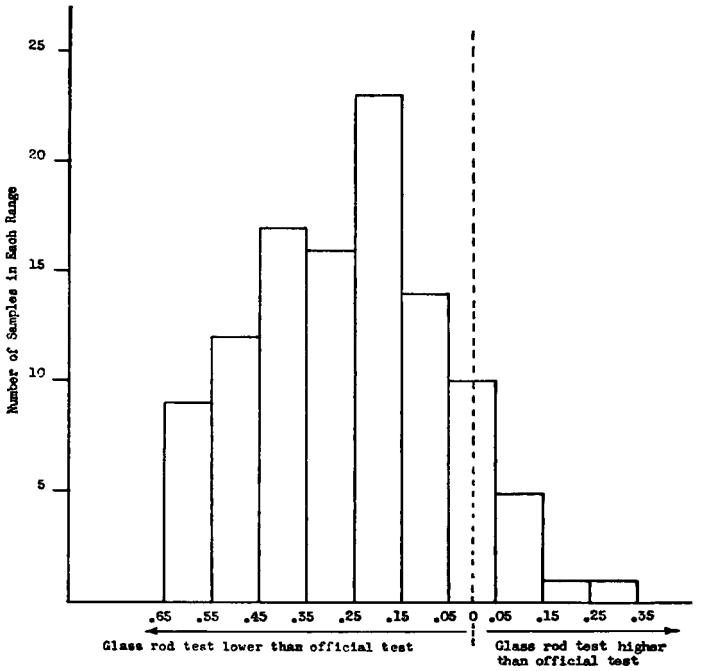

FIG. 1.—Comparison of results by the official method and the glass rod test on process cheese food.

in two cases the difference ran to 0.8 per cent. In every case the glass rod residue was lighter in color and had no caramel odor.

Process American Cheese.—Process American cheese gave a somewhat different picture. Inasmuch as there is no burning as in the case of cheese food, the glass rod tests agree quite closely with the official test. Nearly 50 per cent of the samples agreed within 0.1 per cent and 85 per cent agreed

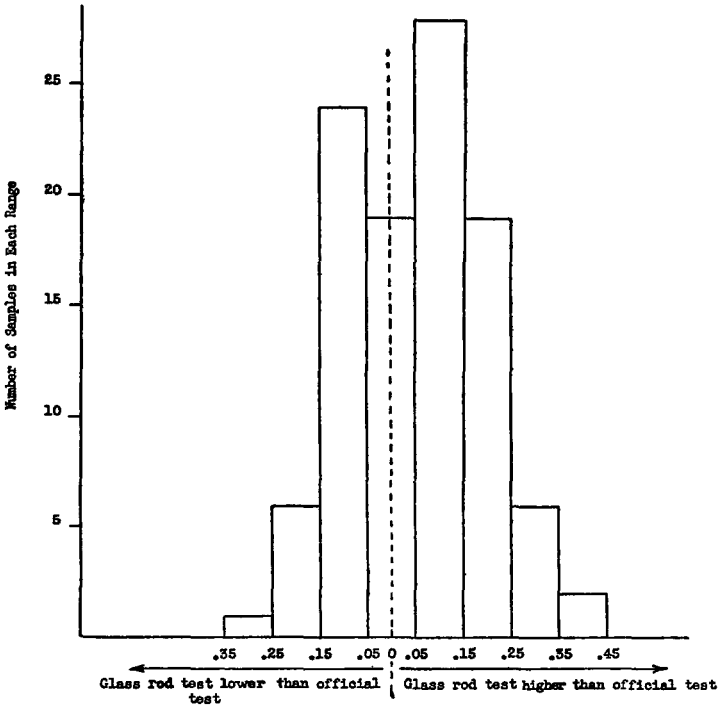

FIG. 2.—Comparison of results by the official method and the glass rod test on American Process Cheese.

within 0.2 per cent. No samples showed a greater difference than 0.4 per cent. Another point noted was that while cheese food nearly always gave a lower result by the glass rod method, process American cheese tended to show slightly higher results by this method. (In 62 of the 105 samples the glass rod test was higher than the official test. Fig. 2.) Reheating the residues of process American cheese dried by the glass rod method led to slight changes, but 88 per cent of the samples did not lose more than 0.2 per cent in weight after an additional three hours of drying.

The glass rod test seems to be especially well adapted to samples of American cheese that do not melt readily on the steam bath. While the official method nearly always yields a low result, the glass rod technique

is capable of removing the water present. Some typical results may be cited to illustrate this: On a 5 lb. loaf of process American, six tests were run by the A.O.A.C. method and six by the glass rod technique. The average of the A.O.A.C. tests was 38.7 per cent, and each test ranged from 38.2 to 38.9 per cent, a spread of 0.7 per cent. The glass rod tests averaged 39.3 per cent and all ran between 39.2 and 39.3 per cent.

Precision of the A.O.A.C. method as compared with the Glass Rod Method.—One of the outstanding advantages of the glass rod technique is an increase in precision. Twenty tests were run by both methods on a single loaf of process American cheese. Using the glass rods, a standard deviation of .065 was obtained, while the official tests gave a standard deviation of 0.290. Twenty-four tests were made by one operator, and twenty-four by another, on a five pound block of white American process cheese. The glass rod technique was used in both cases and standard deviations were 0.14 and 0.18.

Similar studies were made on process cheese food. Twenty determinations were made on each of two 2 lb. loaves by the glass rod method, and twenty more on each of two additional 2 lb. loaves by the official method. The standard deviations obtained for the glass rod technique were 0.042 and 0.093, and for the A.O.A.C. method 0.28 and 0.143.

Rate of Loss of Moisture.—No adequate explanation has been advanced to account for the lower moisture values by the glass rod method other than that the A.O.A.C. test is removing something other than water. This is substantiated by the dark color and caramel odor of the A.O.A.C. test residues. Since none of the dried residues seemed to be affected by additional heating it would seem likely that the presence of water would be conducive to this burning and excess loss of weight. Some tests were run by both methods, and the rate of loss of moisture studied over the five hour period. It was found that the glass rod test samples lost moisture somewhat more rapidly over the first hour than did the A.O.A.C. test samples. At the end of the third hour, both had lost approximately the same amount, but the glass rod tests were levelling off, while the A.O.A.C. test samples were still losing weight to a more marked extent. From the third to the fifth hour, the glass rod tests showed little loss while the A.O.A.C. test samples lost from 0.2 to 0.4 per cent. (Fig. 3).

Effect of Glass on the Test.—The mere presence of the glass rod in the pan does not seem to have any effect on the results. Some tests were run with samples weighed out and a glass rod pressed into the cheese food, but without smearing. The samples showed burning, caramel odors, and a high moisture value. The same tests were repeated and glass beads were pressed into the samples, but no smearing was done. Again the residues were dark and the moisture values higher than those when the same sample was smeared by the glass rod technique.

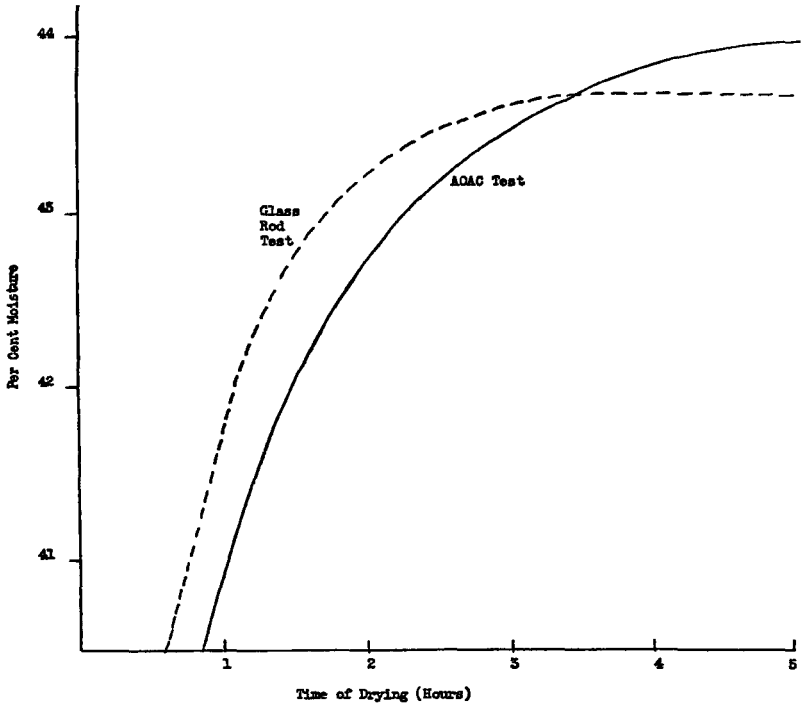

FIG. 3.—Rate of loss of moisture of process cheese food by the A.O.A.C. and glass rod test.

SUMMARY

1. The glass rod technique of smearing the sample results in an improvement in the precision of the vacuum oven method for drying both process cheese and cheese food.

2. The glass rod method yields moisture values that are slightly higher than the official method when applied to process American cheese. In the case of process cheese food, the average result by the glass rod method is lower by about 0.3 per cent, but continued heating of the residues indicates that all the moisture has been removed. In addition, caramel odors and indications of burning are eliminated.

3. It appears that if the moisture is removed rapidly from process cheese food over the first hour, less burning results. Smearing the sample over the dish is conducive to this condition.

AN EVALUATION OF FIVE PROCEDURES FOR THE DETERMINATION OF INTERNAL INSECT INFESTATION OF WHEAT

I. BERBERINE SULFATE FLUORESCENT STAIN FOR WEEVIL EGG PLUGS (IN WHEAT AND CORN)

By G. L. Reed (Baltimore, Md.,) and Kenton L. Harris (Washington, D. C.) (Food and Drug Administration, Federal Security Agency)

The problem of internal insect infestation of grain was discussed in the study reported by Harris, *et al.* (1). As part of this investigation, tests were made on the effectiveness of the methods currently available for the detection of insect infestation within the kernels. Two of these methods, that of Milner, Barney, and Shellenberger (2) and that of Apt (3), were briefly compared with the cracking-flotation and fuchsin stain tests (4). This part of the discussion deals with the berberine sulfate test of Milner, *et al.*

The fuchsin stain method (5) as now used by the Food and Drug Administration consists of immersing the grain for five minutes in water and following with a three minute immersion in a weak acetic acid solution of fuchsin dye. The seeds are then washed with water to remove the excess stain and examined under a low power (10×) wide field microscope.

For this study, 200 kernel portions were removed from each original sample and then treated with berberine sulfate according to the methods of Milner *et al.* (2). The positive kernels showing the fluorescent spots and the negative kernels were separated, thoroughly washed to remove the berberine sulfate and then stained with fuchsin. All the kernels that were found positive by both the berberine sulfate and the fuchsin stain and those found positive by the fuchsin stain alone were dissected under low magnification and examined for eggs and larvae. If an egg plug was present, all kernels that were positive to berberine sulfate were also positive to fuchsin.

Although the berberine sulfate method stated that the alkaloid stained weevil egg plugs, it was hoped that the method could be adapted to reveal the total damage resulting from both weevil and grain moth infestation. An examination of several samples of both wheat and corn revealed that the alkaloid was selective and stained only the weevil egg plugs. Fuchsin and other biological stains will reveal insect feeding punctures. Five corn and three wheat samples which were moth damaged (moth damage ranged from 2–25%), were examined with berberine sulfate; no damage not visible to the naked eye was revealed by the use of the alkaloid and ultraviolet radiation.

Table 1 reports the results of the examination of corn and wheat samples which had been infested with living adult weevils in the laboratory. Thus the egg plugs were not subjected to any rough handling which might

have caused the plugs to become dislodged as is often the case with commercial samples. (The first six corn samples were, however, taken from corn as encountered in commerce.) Those kernels showing one or more weevil egg plugs were reported as damaged.

TABLE 1.—*Comparison between the berberine sulfate fluorescence, and fuchsin stain methods for the detection of weevil egg plugs in wheat and corn (200 kernels from each sample examined)*

TYPE GRAIN	SAMPLE NUMBER	NUMBER OF KERNELS WITH EGG PLUGS	
		BERBERINE SULFATE	FUCHSIN STAIN
Soft Red Winter Wheat	1	0	3
	2	1	5
	3	2	6
	4	2	9
	2	5	9
	6	8	9
	7	6	11
	8	8	23
	9	15	29
	10	14	30
	11	33	45
	12	56	63
White Corn†	1	0	5
	2	0	7
	3	0	7
	4	0	9
	5	0	12
	6	0	13
	7*	6	9
	8*	7	12
	9*	11	14
	10*	11	16

* These four corn samples were individually prepared by adding damaged kernels from an almost 100% damaged sample to each undamaged sample. The damaged kernels had a minimum of five egg plugs per kernel.

† One sample of yellow corn was examined, but not included in the tabulation. This sample showed approximately 25% damage due to weevil egg plugs by the fuchsin stain method, but apparently the yellowish-red color of the corn masks the yellow fluorescence in berberine sulfate method as no damage was observed.

As a further check, damaged kernels were examined under a low power (10×) wide field microscope by dissection for the larvae or egg. It was found that with few exceptions the wheat kernels showing fluorescent spots contained either larvae or an egg. However, corn often gives a fluorescence around the germ which is misleading. For example, the last four samples in the tabulation had an average of two kernels which were assumed to be damaged, but damage could not be confirmed by dissection of the kernel. All of these samples had at least one kernel in this category with the number running as high as four in one sample.

CONCLUSIONS

1. Berberine sulfate is a selective dye and will not show moth damage, weevil feeding punctures, or egg cavities from which the cap has been dislodged.

2. Even when the egg plug is present, the fluorescence produced in the berberine sulfate method is either masked by other fluorescent substances or the alkaloid does not stain, thus causing low results. (It was not uncommon to see a weevil exit hole and yet be unable to find an egg plug by the berberine sulfate method while later examination reveals several egg plugs by using the fuchsin stain.)

3. Fluorescence around the germ by the berberine sulfate method on corn cannot always be distinguished from fluorescence due to the egg plug. This is also true of wheat but to a lesser degree.

4. The method was designed for and recommended as a test for weevil egg plugs, and not as a means of measuring general insect infestation or damage. Its rapid application (3–5 minutes) might make it useful as a means of sorting out samples which give positive results. However, since the fuchsin stain does not give false positives and also shows egg plugs in greater numbers, the berberine sulfate method offers no advantages over the fuchsin stain.

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AN EVALUATION OF FIVE PROCEDURES FOR THE DETERMINATION OF INTERNAL INSECT INFESTATION OF WHEAT

II. GELATINIZATION IN SODIUM HYDROXIDE

By GEORGE E. KEPPEL (Minneapolis, Minnesota) and KENTON L. HARRIS (Washington D. C.) (Food and Drug Administration, Federal Security Agency)

The method of Apt (1) mentioned in Part I of this paper was also investigated and a cursory report has been made (2). The following is a detailed report on the evaluation of this test.

The sodium hydroxide treatment consists in boiling 5 grams (about 100 kernels) of wheat in 50 ml of 10 per cent sodium hydroxide for 10

minutes. This results in a gelatinization and clearing of the starch so that the kernels become translucent and it is possible to see insects within the kernels. After clearing, the sodium hydroxide is drained off and the gelatinized kernels washed with distilled water. The kernels are then transferred to a Petri dish, covered with water, allowed to soak for 5 minutes and examined under a low power binocular microscope to detect internal insect infestation.

In a preliminary trial, the method was applied to the 18 wheat samples received from May 17 to June 8, 1950, as part of the investigation of contamination of wheat and flour. No infestation was detected in any of these samples. Subsequent examinations of portions of the same samples by the cracking test (3) showed weevils and flat grain beetles in one sample, flat grain beetles in six, and nothing in the remaining eleven. The fuchsin stain test (3) showed weevil egg plugs and/or feeding punctures in two samples, insect cutting or tunneling in three samples and nothing in thirteen. The hydroxide test was later applied only to those samples received during the course of the survey that showed some evidence of weevil infestation by the cracking-flotation or fuchsin stain test. Of the six such samples received from June 9 to October 17, none showed infestation by the hydroxide method.

At this point it appeared desirable to test the method with wheat showing more infestation than that being routinely collected in the Minneapolis area during the survey, and cultures were prepared by adding weevils to wheat showing no infestation and allowing them to multiply. Two cultures were incubated and duplicate samples were withdrawn at varying intervals and examined by the fuchsin stain* and hydroxide tests. The results are tabulated in Table 1. The greater sensitivity of the fuchsin stain test is revealed by the greater number of insect damaged kernels shown by this test.

Further tests were run to find out if the method could detect infestation in naturally infested wheat samples containing weevils in all stages of development. Ten portions from each of two samples were examined by the fuchsin stain test and ten additional duplicate portions were examined by the hydroxide test. A complete analysis was made as in Table 1. In Table 2 the results are summarized, by averages only.

The data summarized in Table 2 substantiate the earlier work and show that the sodium hydroxide gelatinization procedure is not as sensitive as the stain test for the determination of internal infestation of wheat. However, it must be kept in mind that the two methods measure entirely different effects. The staining detects egg deposits and, to some extent, evidence of feeding by weevils and other insects. The hydroxide method detects only larvae (after they are fairly well developed), pupae, and adults.

* The fuchsin stain was chosen as the basis of comparison, since both it and the hydroxide test involve a sorting of infested or damaged kernels.

TABLE 1.—*Progressive infestation of sound wheat containing added weevils as measured by Fuchsin stain and sodium hydroxide tests (Each Test on 100 Kernels)*

	CULTURE NO. 1	NUMBER DAYS' INCUBATION																			
		1					2					5					6				
		Test 1	Test 2	Test 3	Test 4	Average	Test 1	Test 2	Test 3	Test 4	Average	Test 1	Test 2	Test 3	Test 4	Average	Test 1	Test 2	Test 3	Test 4	Average
Fuchsin Stain	Number kernels with weevil egg puncture	0	1	0		0.3	0	0	0			0	0	0			1	1	1		1.0
	Number weevil egg puncture.	0	1	0		0.3	0	0	0			0	0	0			2	1	1		1.3
	Number kernels with weevil feeding puncture	1	1	0		0.7	0	0	0			1	0	0		0.3	0	1	0		0.3
	Number weevil feeding puncture	1	1	0		0.7	0	0	0			1	0	0		0.3	0	1	0		0.3
	Kernels with cutting or tunneling	0	0	1		0.3	1	0	0		0.3	1	1	2		1.3	2	1	1		1.3
	Total insect damaged kernels	1	2	1		1.3	1	0	0		0.3	2	1	2		1.7	3	3	2		2.7
	Total units insect damage	1	2	1		1.3	1	0	0		0.3	2	1	2		1.7	4	3	2		3.0
Hydroxide	Number kernels with insect larvae	0	0	0		0	0	0	0		0	0	1	0		0.3	0	0	0		0
	Number larvae	0	0	0		0	0	0	0		0	0	1	0		0.3	0	0	0		0

	CULTURE NO. 2	NUMBER DAYS' INCUBATION																			
		3					6					10					87				
		Test 1	Test 2	Test 3	Test 4	Average	Test 1	Test 2	Test 3	Test 4	Average	Test 1	Test 2	Test 3	Test 4	Average	Test 1	Test 2	Test 3	Test 4	Average
Fuchsin Stain	Number kernels with weevil egg puncture	0	1	0	0	0.2	1	0	0	2	0.8	0	1	0	1	0.5	5	7	5	8	6.3
	Number weevil egg puncture	0	1	0	0	0.2	1	0	0	2	0.8	0	1	0	1	0.5	5	7	5	8	6.3
	Number kernels with weevil feeding puncture	0	0	0	0	0	0	1	2	0	0.8	2	1	0	0	0.8	3	3	3	0	2.3
	Number weevil feeding puncture	0	0	0	0	0	0	1	3	0	1.0	2	1	0	0	0.8	4	4	5	0	3.3
	Kernels with cutting or tunneling	0	0	0	0	0	1	0	0	0	0.2	0	0	2	0	0.5	1	1	0	2	1.0
	Total insect damaged kernels	0	1	0	0	0.2	2	1	2	2	1.8	2	2	2	1	1.8	8	11	8	10	9.3
	Total units insect damage	0	1	0	0	0.2	2	1	3	2	2.0	2	2	2	1	1.8	10	12	10	10	10.5
Hydroxide	Number kernels with insect larvae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	1	1.5
	Number larvae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	1	1.5

Other evidence of insect damage, such as feeding punctures, is destroyed by the alkali digestion. Occasionally a portion of a tunnel may be seen, but in such an instance the larvae is usually seen as well.

Defects in the method may be summarized as follows: (1) Kernels are made only partially translucent. The bran in the crease is not removed. Portions of remaining bran adhere in loose shreds and obscure translucency. About one-third of the endosperm remains ungelatinized. (2) The incomplete translucency necessitates the turning and manipulation of each

TABLE 2.—*Comparison of findings by stain and hydroxide tests on replicate samples of wheat (Each test on 100 kernels)*

TEST	TYPE OF CONTAMINATION	AVERAGE OF 10 TESTS ON SAMPLE #1	AVERAGE OF 10 TESTS ON SAMPLE #2
Stain	Total number insect damaged kernels	5.5	7.7
	Total number units insect damage	6.1	10.7
Hydroxide	Number kernels with larvae	0.5	1.9
	Total number of larvae	0.5	1.9
	Number kernels with adults	0.1	0
	Total number of adults	0.1	0
Repeat Hydroxide	Number kernels with larvae	0.3	No test
	Total number larvae	0.3	No test

kernel under the microscope. Because of the treatment, the kernels have a jelly-like texture and examination is necessarily slow.

Modifications which were tried in an attempt to resolve some of the difficulties are summarized below:

1. Preliminary bleaching in acid sodium sulfite or hydrogen peroxide causes a clouding after the alkali is added.

2. Treatment with nitric acid, then sulfuric acid, and then a water rinse will remove the bran except that in the crease, but the treatment also results in some clouding of the endosperm when the grain is treated later with the alkali. However, the acid treatment might be used as a starting point for other types of examination.

3. Tests with transmitted light, colored light, and various degrees of magnification showed the superiority of reflected white light and 9 to 12× magnification.

4. Visibility was improved slightly by pressing the kernels between glass plates, but the technique was messy.

CONCLUSIONS

The sodium hydroxide method appeared useful in detecting internal infestation of wheat in the more advanced stages. The method failed to

detect beginning or early stages of weevil infestation. Although further development work on the method might have resulted in improving its sensitivity, convenience, and simplicity to a point where it would be useful as an auxiliary test to the staining method, such development was beyond the scope of this work, and in the absence of promising results the work was discontinued.

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AN EVALUATION OF FIVE PROCEDURES FOR THE DETERMINATION OF INTERNAL INSECT INFESTATION OF WHEAT

III. BUHLER MILLING OF THE WHEAT*

By J. FRANK NICHOLSON, KENTON L. HARRIS, FRANK R. SMITH, and MARYVEE G. YAKOWITZ (Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The report on contamination of wheat and wheat flour (1) mentioned the use of a Buhler pilot mill as a means of evaluating insect contamination in wheat ready for milling. This approach attacks the problem of grain contamination in terms of the ultimate fragment count of the flour rather than directly assessing contamination of the wheat. This particular study involved a comparison of the calculated straight flour (2) of a commercial mill with the straight 70 per cent extraction made on a Buhler mill. The Buhler mill had been adjusted and sieved by the milling company for their usual flour quality determinations.

Wheat and flour samples were collected from the wheat and flour streams in the commercial mill and analyzed as described by Harris, *et al.* (3). In addition, an approximately 20-pound cleaned wheat sample was taken from the commercial mill, milled on the Buhler, and the resulting flour analyzed. Analyses of the commercial and Buhler flours were made independently in the Food and Drug Administration's field district laboratory and in the Division of Microbiology of the Food and Drug Administration. Analyses of the flours were also made by the firm's laboratories, but only the Food and Drug analyses are reported here (Table 1). In a few cases the table also contains data on the flour produced by the milling of comparable aliquots of the wheat on the U. S. Department of Agriculture's Buhler mill.

The results clearly indicate that analyses of the flour produced by a

* This work was suggested by and carried out with the cooperation of a mill that prefers to remain anonymous.

TABLE 1.—*Comparison of insect fragment count on commercially milled and Buhler milled flour*

TYPE OF MILL	TYPE OF FLOUR	SAMPLE 1			SAMPLE 2			SAMPLE 3			SAMPLE 4			SAMPLE 5			SAMPLE 6		
		LAB 1	LAB 2	PER CENT*	LAB 1	LAB 2	PER CENT	LAB 1	LAB 2	PER CENT	LAB 1	LAB 2	PER CENT	LAB 1	LAB 2	PER CENT	LAB 1	LAB 2	PER CENT
Commercial	Patent	24	22	60.9	108	122	63.6	29	45	63	6	3	58.5	3	3	55.4	3	2	60.4
	1st Clear	50	35	28.1	205	193	27.2	84	107	27.3	9	8	28.9	6	8	31.2	7	5	27.8
	2nd Clear	58	60	11.1	239	281	9.2	170	178	9.7	30	33	12.6	8	11	13.4	7	4	11.8
	Calculated Straight	35	30		146	156		58	75		10	8		5	6		5	3	
Buhler	Straight	21			168			51			10			8			4		

TYPE OF MILL	TYPE OF FLOUR	SAMPLE 7			SAMPLE 8			SAMPLE 9			SAMPLE 10			SAMPLE 11			SAMPLE 12		
		LAB 1	LAB 2	PER CENT	LAB 1	LAB 2	PER CENT	LAB 1	LAB 2	PER CENT	LAB 1	LAB 2	PER CENT	LAB 1	LAB 2	PER CENT	LAB 1	LAB 2	PER CENT
Commercial	Patent	8	2	62.1	6	2	60.4	7	10	59.9	23	26	57.5	10	8	59.8	11	11	59.8
	1st Clear	2	4	24.5	10	6	25.4	19	14	28.5	35	32	30.0	26	26	28.1	29	25	27.0
	2nd Clear	16	4	13.4	20	20	14.2	20	19	11.6	65	72	12.5	38	43	12.1	46	47	13.2
	Calculated Straight	7	3		8	6		11	12		32	34		18	17		20	20	
Buhler	Straight	6			4			9			26			20			23		
Buhler 2	Straight										27			18			27		

* Percentages refer to per cent extraction of each of the three types of flour and were used in calculating the "calculated straight" flour.

pilot mill can be used to mill wheat and predict the insect fragment count of the flour when the same wheat is milled on a commercial mill.

REFERENCES

(1) *This Journal*, 35, 155 (1952).
(2) *Ibid.*, 35, 124 (1952).
(3) *Ibid.*, 35, 115–158 (1952).

AN EVALUATION OF FIVE PROCEDURES FOR THE DETERMINATION OF INTERNAL INSECT INFESTATION OF WHEAT

IV. VISUAL EXAMINATION FOR INSECT EXIT HOLES

By J. Frank Nicholson (Washington, D. C.), John C. Akers (Kansas City, Missouri), Kenton L. Harris and O. L. Kurtz (Washington, D. C.) (Food and Drug Administration, Federal Security Agency)

There is great need for a rapid sorting procedure, which can be used by non-technical personnel without extensive laboratory facilities, to evaluate the amount of internal insect contamination of wheat.

The wheat and flour survey (1) contained a method based upon the visual examination of grain. However, this method was qualitative, not quantitative, and some evidence of infestation often was reported when little or no internal infestation could be detected by other methods. Further work was done on 173 survey samples to see if these difficulties could be overcome by standardizing on a definitive type of damage which is an index of internal infestation and which does not, by imperceptible gradations, shade off into types of cutting caused by surface-feeding insects. Although surface-feeding insects do damage the kernels, they are internal insects only as secondary invaders. This surface feeding or cutting through to the opposite surface from old cavities may range from rather large cuts to microscopic nibblings, with all gradations in between. Since these surface-feeding insects do not materially contribute to the insect fragment count of the flour, and since the problem is to devise a rapid macroscopic procedure to evaluate internal damage of wheat, it was felt that the type of damage to be counted should be limited to that caused by insects feeding within the kernels and yet which was conspicuous enough to be readily visible on the surface. It was felt that insect exit holes might meet these requirements.* The examination for exit holes and windows is as follows:

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* The use of exit holes was suggested by J. L. Trawick, U. S. Food and Drug Administration, Washington, D. C.

Weigh out 100 g of wheat from the well-mixed sample. Examine a small amount at a time, under good light, by rolling the grain across a piece of fine sandpaper with a knife or spatula. Count the number of grains showing either an insect exit hole or a "window." In the case of an exit hole, the full extent of the boring cannot usually be seen through the hole. The term "exit hole" should be restricted to rounded holes with smooth edges. Do not count kernels with only surface cutting when the bottom is plainly visible, or with tunnels which penetrate straight through from one side of the kernel to the other with little change in diameter. Exit holes should not be confused with entrance holes, which are smaller in diameter. (Exit holes usually have a minimum diameter of about 1/32 of an inch.) Report the number of kernels containing one or more exit holes or windows. This is a rapid examination and takes not over 15 minutes.

The 173 samples† were selected from the 1410 survey samples on the basis of degree and type of internal infestation, geographical origin, and type of wheat. Duplicate 100 gram portions were separated from a well-mixed sample using a Jones sample divider, and comparisons were made with results obtained by the cracking-flotation test (2).

Table 1 gives the results on individual samples by the cracking-flotation and exit hole tests, and also lists data on the X-ray tests which will be discussed in Part V of this paper. The complete listing is given so that comparisons can be made on individual samples. The table is arranged in increasing order of infestation by the cracking test (column 1). Columns 2 and 3 give the counts on individual 100 gram subdivisions of the original samples, and the average of the two examinations is given in column 4. The correlation coefficient between the cracking test and the individual visual exit hole counts is 0.73 for both counts; to the average of the two counts it is 0.75. The ratio of total insects by cracking-flotation to total number of kernels with exit holes is 4.5 to 1.

The data in Table 1 have been re-arranged in Table 2 in ascending order of infestation by the average visual exit hole test. The comparable counts by the cracking-flotation tests are shown in column 4. There is a progressive rise in the average number of internal insects, as demonstrated by the cracking test, with the increase in the number of exit holes. Above the 4.5–5.0 exit hole range there are so few samples in each category that the trend is more irregular.

The exit hole test appears promising as a rapid sorting procedure for detecting internal infestation in wheat.

REFERENCES

(1) *This Journal*, 35, 115 (1952).
(2) *Ibid.*, 35, 121 (1952).

(*See pp. 148, 149, 150 for Tables 1 and 2.*)

† Statistically 200 samples were chosen. Because of sample contamination and other factors only 173 were finally used.

TABLE 1.—*Results on 173 samples by cracking flotation, by visual examination for for insect exit holes, and by X-ray*
(100 gram samples)

INSECTS BY CRACKING-FLOTATION	NUMBER OF KERNELS WITH EXIT HOLES			DAMAGED KERNELS BY X-RAY EXAM.		INSECTS BY CRACKING-FLOTATION	NUMBER OF KERNELS WITH EXIT HOLES			DAMAGED KERNELS BY X-RAY EXAM.	
	SAMPLE 1	SAMPLE 2	AV.	GROSS DAMAGE	EXIT HOLES		SAMPLE 1	SAMPLE 2	AV.	GROSS DEMAGE	EXIT HOLES
0	0	0	0	2	0	6	1	2	1.5	16	5
0	1	0	0.5	13	2	6	0	1	0.5	16	2
0	0	0	0	2	0	6	0	1	0.5	14	4
						6	0	1	0.5	10	1
1	0	0	0	6	1	6	6	4	5	19	6
1	0	0	0	4	0	6	5	2	3.5	26	6
1	0	0	0	5	0						
1	1	0	0.5	6	1	7	2	1	1.5	13	4
1	0	0	0	2	0	7	0	0	0	14	1
1	0	0	0	5	0	7	3	5	4	23	3
1	0	0	0	5	0	7	0	0	0	7	0
1	0	0	0	8	0	7	2	2	2	15	3
1	0	0	0	3	1	7	0	2	1	17	5
1	0	0	0	4	0	7	1	1	1	11	2
						7	1	2	1.5	11	2
2	2	2	2	10	4	7	1	1	1	9	2
2	0	1	0.5	7	0						
2	0	0	0	7	0	8	0	0	0	12	1
2	0	0	0	7	0	8	0	1	0.5	14	5
2	0	0	0	9	2	8	1	0	0.5	23	5
2	0	1	0.5	3	0	8	6	4	5	27	7
2	0	0	0	4	0	8	1	2	1.5	9	3
2	2	3	2.5	6	1	8	1	2	1.5	9	2
2	0	0	0	4	0	8	0	0	0	7	0
2	1	1	1	9	2						
						9	7	3	5	24	14
3	2	0	1	6	1	9	0	1	0.5	21	6
3	1	1	1	14	3	9	5	8	6.5	23	8
3	0	1	0.5	13	3	9	0	0	0	8	1
3	2	1	1.5	16	4	9	3	1	2	15	5
3	0	0	0	5	1						
3	0	0	0	11	0	10	2	1	1.5	15	2
3	0	0	0	9	2	10	0	2	1	16	4
						10	2	2	2	30	10
4	0	1	0.5	12	3	10	1	0	0.5	10	1
4	1	3	2	16	3						
4	0	3	1.5	8	2	11	3	1	2	25	10
4	1	3	2	10	3	11	1	2	1.5	11	1
4	0	1	0.5	11	3	11	5	5	5	25	7
4	0	0	0	6	1	11	3	1	2	22	12
4	0	0	0	6	1	11	1	2	1.5	20	4
4	0	0	0	16	2	11	4	5	4.5	21	8
4	0	0	0	7	2	11	0	2	1	15	2
5	1	2	1.5	14	3	12	2	3	2.5	38	13
5	2	1	1.5	12	3	12	3	2	2.5	17	6
5	3	2	2.5	14	3	12	2	3	2.5	15	3
5	0	1	0.5	9	1	12	2	3	2.5	20	10
5	0	0	0	7	0	12	3	6	4.5	31	9
5	0	0	0	10	2						
5	0	2	1	10	1	13	2	0	1	36	12
5	1	2	1.5	19	6	13	0	0	0	15	3
5	0	1	0.5	8	2	13	0	0	0	13	2
5	5	3	4	16	3	13	1	0	0.5	12	1
5	0	0	0	5	0	13	3	3	3	14	2
5	0	0	0	2	0	13	0	6	3	20	3

TABLE 1.—(*continued*)

INSECTS BY CRACKING-FLOTATION	NUMBER OF KERNELS WITH EXIT HOLES			DAMAGED KERNELS BY X-RAY EXAM		INSECTS BY CRACKING-FLOTATION	NUMBER OF KERNELS WITH EXIT HOLES			DAMAGED KERNELS BY X-RAY EXAM.	
	SAMPLE 1	SAMPLE 2	AV.	GROSS DAMAGE	EXIT HOLES		SAMPLE 1	SAMPLE 2	AV.	GROSS DAMAGE	EXIT HOLES
13	1	2	1.5	10	4						
13	4	3	3.5	18	5	23	2	5	3.5	20	5
13	0	3	1.5	15	3	23	4	4	4	23	7
14	4	4	4	22	8	24	1	3	2	33	3
14	2	5	3.5	14	5						
14	10	7	8.5	31	12	25	26	23	7.5	67	30
14	2	3	2.5	23	5	25	12	23	17.5	35	14
14	1	0	0.5	18	1	25	6	3	4.5	25	10
14	4	1	2.5	13	1						
14	3	2	2.5	21	6	26	0	2	1	22	3
14	1	4	2.5	22	7	26	3	0	1.5	29	9
						26	6	8	7	43	13
15	3	2	2.5	16	8	26	10	15	12.5	65	19
15	2	0	1	16	2	26	10	11	10.5	54	14
15	1	3	2	18	6						
15	2	3	2.5	24	5	27	1	0	0.5	19	4
15	4	1	2.5	23	6	27	12	8	10	34	10
16	0	0	0	18	4	28	5	5	5	30	10
16	8	9	8.5	32	9	28	5	10	7.5	56	14
16	5	3	4	22	8	28	3	3	3	24	4
17	4	8	6	22	7	30	11	8	9.5	48	19
17	0	0	0	16	3						
						31	4	5	4.5	34	13
18	6	3	4.5	44	12	31	8	5	6.5	36	13
18	3	4	3.5	23	3						
18	3	4	3.5	29	9	34	22	16	19	80	37
18	3	4	3.5	16	4	34	4	0	2	22	6
18	1	4	2.5	27	3						
18	3	3	3	24	8	35	3	4	3.5	43	13
18	1	1	1	18	3						
18	5	3	4	24	6	46	8	8	8	50	16
19	4	5	4.5	37	8	47	8	12	10	53	24
19	1	0	0.5	21	2						
19	1	3	2	28	4	48	5	6	5.5	57	15
19	2	3	2.5	19	5						
						52	7	7	7	55	18
20	2	5	3.5	34	11						
20	4	3	3.5	24	3	55	13	13	13	70	23
20	3	7	5	42	12						
						78	9	13	11	76	19
21	3	4	3.5	34	8						
21	4	8	6	28	12	91	52	34	43	140	62
21	5	5	5	19	3						
						119	35	29	15	112	39
22	8	9	8.5	46	19						
22	6	4	5	34	8	Totals					
22	3	3	3	25	6	2485	545	567	556	3761	1057
22	6	4	5	19	7						

TABLE 2.—*Relationship between visual exit holes and insects in the wheat* (100 gram samples)

AVERAGE VISUAL* EXIT HOLES	NUMBER OF SAMPLES	INSECTS BY CRACKING	
		RANGE	AVERAGE
0	36	0–16	4.5
0.5– 1.0	33	0–27	8.4
1.5– 2.0	28	2–34	10.4
2.5– 3.0	20	2–28	14.4
3.5– 4.0	17	6–35	17.0
4.5– 5.0	15	8–31	17.5
5.5– 6.0	3	17–48	28.7
6.5– 7.0	4	9–52	29.5
7.5– 8.0	3	25–46	33.0
8.5– 9.0	3	16–77	38.3
9.5–10.0	3	27–47	34.7
10.5–11.0	2	26–78	52.0
11.5–12.0	0		
12.5–13.0	2	26–55	4.05
15	1		119
17.5	1		25
19.0	1		34
43.0	1		91

* The use of two subdivisions often results in a 0.5 remainder which for convenience has been included with the next higher integral number.

AN EVALUATION OF FIVE PROCEDURES FOR THE DETERMINATION OF INTERNAL INSECT INFESTATION OF WHEAT

V. THE USE OF X-RAYS

By J. FRANK NICHOLSON (Food and Drug Administration, Washington, D. C.), MAX MILNER (Kansas State College, Manhattan, Kansas), W. H. MUNDAY (Food and Drug Administration, Kansas City, Missouri), O. L. KURTZ, and KENTON L. HARRIS (Food & Drug Administration, Federal Security Agency, Wash. D. C.)

In recent years there has been an increased effort to determine the amount of internal insect infestation in cereal grains. A new approach to this problem was reported by Milner, Lee, and Katz (1, 2) who used radiographic techniques for the successful X-ray testing of wheat. Milner (3) has surveyed the developments of methods for detecting this infestation and has discussed the apparent possibilities of the X-ray method. Recently, Milner, Lee, and Katz (4) re-evaluated the X-ray procedure and showed the possibilities of obtaining radiographic information on various grains and seeds.

The wheat survey conducted by the U. S. Food and Drug Administration (5) made use of the methods then available for the evaluation of internal infestation and stated (6) that further work on the testing of seselected survey samples by the X-ray technique was being carried out with Dr. Max Milner of Kansas State College. The present paper reports the results of that investigation and the interpretation of radiographic findings in the 173 wheat samples listed in part IV, above.

As is the case with any radiographic technique, it is necessary to be able to interpret the radiographic image in terms of the internal damage being studied. Since the internal damage of wheat shows as shadows on the radiograph, kernels showing suspect shadows were dissected and the cause of the radiographic image was determined. Such a method was found to be necessary to interpret the radiographs in terms of actual insects and insect damage, and to correlate the radiographic techniques to other methods of insect detection. To facilitate this examination, kernels were mounted with an adhesive on paper before making the radiograph so that the finished radiograph could be examined with an X-ray illuminator and the image compared to the kernels fixed to the paper mount. Suspect individual kernels were dissected and examined under low-power magnification. From experience gained by this procedure, it was possible to differentiate on the radiograph between the various gradations of insect damage, and seed characteristics which resembled insect damage. Those seed characteristics giving rise to suspicious shadows on the radiograph are illustrated and described in Figure 1. Types of insect damage in the kernels shown on the radiographs are illustrated and described in Figure 2.

Live larvae, pupae, and, to a much less extent, adults can be seen and identified as such. Although dead larvae can be seen and identified as larvae, dead adults and pupae may produce no shadow on the radiograph. The visibility of insects on the radiograph apparently depends to a large extent on the amount of water present.

Examination of radiographs for total insect damage, including the most minute, was too time consuming to be practical. A radiographic examination of 100 g. of wheat for all gradations of insect damage involved the precise scanning of over 3000 kernels and took a minimum of one to one and one-half hours. Moreover, dehydrated insects were not visible, and multiple damage was difficult or impossible to determine as such. More insect damaged kernels were observed on the radiographs and dissected from the mounted kernels than were recovered by the cracking-flotation test, which has already been established as a reliable laboratory procedure. It was concluded that there would be little value in using additional criteria simply to increase the numerical value of the results. Hence an attempt was made to correlate only the more readily visible (grossly) insect damaged kernels with the cracking-flotation results. The initial

approach in this direction was an attempt to establish a relationship between the exit holes on the radiograph and the exit holes as determined by the visual examination.

Using the criteria discussed in Figure 2, radiographs of the 173 samples were examined for exit holes. Table 1, Part 4, gives the individual results obtained. It is evident from this table that exit holes as judged by the radiographs exceed those found by rapid visual examination.

Table 1 (below) regroups the individual exit hole results (visual and

TABLE 1.—*Number of exit holes by visual examination compared with exit holes by the X-ray technique (100 g samples)*

VISUAL EXIT HOLES*	NUMBER OF SAMPLES	X-RAY EXIT HOLES	
		RANGE	AVERAGE
0	36	0– 4	0.8
0.5– 1.0	33	0–12	2.7
1.5– 2.0	28	1–12	4.5
2.5– 3.0	20	1–13	5.3
3.5– 4.0	17	3–13	6.3
4.5– 5.0	15	3–14	8.9
5.5– 6.0	3	7–15	11.3
6.5– 7.0	4	8–18	13.0
7.5– 8.0	3	14–30	20.0
8.5– 9.0	3	9–19	13.3
9.5–10.0	2	10–24	17.7
10.5–11.0	2	14–19	16.5
11.5–12.0	0		
12.5–13.0	2	19–23	21.0
15.0	1		39.0
17.5	1		14.0
19.0	1		37.0
43.0	1		62.0

* Average of two 100 gram aliquots. The use of two portions often results in a 0.5 remainder which for convenience has been included with the next higher integral number.

radiographic). They are arranged in order of increasing number of visual exit holes. There is a corresponding rise in X-ray exit holes until the number of samples in any one group falls off and individual sample variations make the rise more irregular.

Table 1 of Part IV also tabulates the cracking-flotation, exit hole, and X-ray data on the 173 samples. Since the number of exit holes is only an indirect measure of internal insect contamination, it was felt that a more direct measure would be obtained if other insect-damaged kernels could be counted. In order not to lengthen the time spent in reading the radiograph, the examination was limited to the more extensively (grossly) damaged kernels, and took from 5 to 10 minutes. These were compared with the cracking-flotation results.

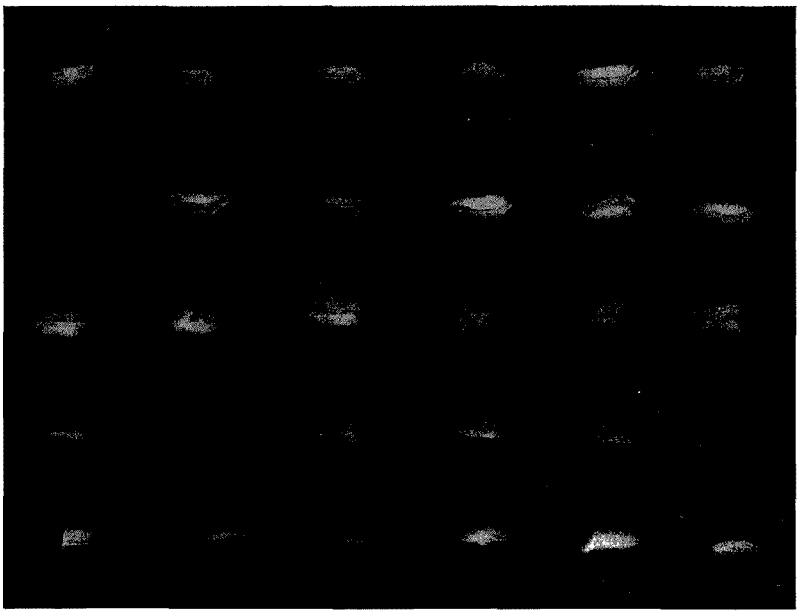

FIG. 1—Imperfections not due to infestation.

1. Receding germ layer. A partial separation of the germ layer from the endosperm giving rise to a darkened, curved streak resembling a comma.
2. Receding of the endosperm from the bran coat of the ventral groove. This condition gives rise to one or two longitudinal tunnels tapering and fusing at each end of the kernel. No break or opening appears throughout the length of the tunnel.
3. Fractures in the endosperm. These may be seen over the entire radiograph of the kernel and appear as dark linear streaks.
4. Depressions on the kernel surface. A diffuse area with variation of the gray intensity.
5. Mechanical breakage. Since the breakage usually occurs along the above fracture lines, one surface at least will have sharply-defined straight edges.

In Table 1, Part IV, note that insects by the cracking-flotation test and the number of grossly damaged kernels are of about the same magnitude. They are summarized in Table 2, which is arranged in order of ascending values of the cracking-flotation test. There is a progressive rise in the damaged kernels shown by X-ray examination which, as a rule, corresponds to the rise indicated in the cracking-flotation results. The correlation coefficient for the cracking flotation test and X-ray for gross insect damage is excellent (.88). For all 173 samples the total X-ray results were 1.5 times that of the cracking-flotation test.

The X-ray technique is a means of directly viewing the contents of each individual wheat kernel without the necessity of using intermediate

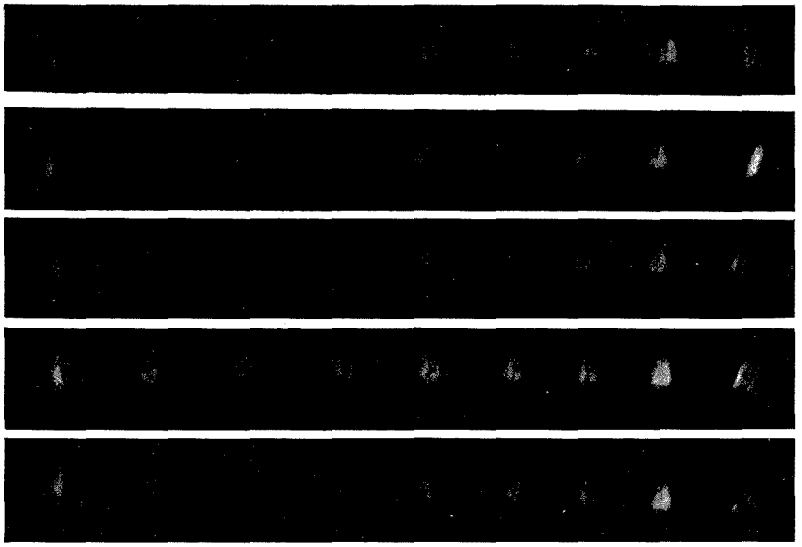

FIG. 2.—Internal damage by insects.

1. Weevil feeding holes, egg plugs, larval entrance holes. This type of damage may appear as a dot, an oval, or a line, depending on the orientation of the wheat kernel. If the tunnel is parallel to the X-ray beam, it will radiograph as a dot. When the damage is perpendicular to the X-ray beam it will show as a straight line. All variations intermediate between these two extremes are possible, giving rise first to a shorter line and later as it approaches the parallel position, an oval form. The intensity of the shadow is inversely proportional to the thickness of the tissue penetrated. Little or no differentiation could be made between weevil feeding, egg plugs, and moth entrance holes.
2. Early tunneling. This type of damage is a continuation of the above and varies from slight to extensive excavations as the larva grows in size. These excavations vary from a series of fine thread-like tunnels to a completely hollowed chamber. The radiographic intensity varies from a very light gray in the case of small tunnels to black from those with extensive damage. Moth damage resembles that of the weevil except for the frassy, granular material remaining in the kernel (not illustrated).
3. More advanced tunneling.
4. Late stages of tunneling.
5. Exit holes (weevil). These appear as dark shadows on the radiograph which represent a large excavation within the kernel and an accompanying intensely dark spot where the tunneling extended through to the surface as an exit hole. With little frass remaining in the cavity, the radiographic shadow appears almost as dark as the background. Granular material within the tunneled kernel is likely to be due to moth larval pellets entangled in the webbing which prevents its sifting out.

TABLE 2.—*Relationship between internal insects by the cracking test and internal insect damage by X-ray gross insect damage*

CRACKING TEST INSECTS/100 GRAMS	NUMBER OF SAMPLES	X-RAY GROSS DAMAGE	
		RANGE	AVERAGE
0	3	2– 13	5.7
1	10	2– 8	4.8
2	10	3– 10	6.6
3	7	3– 16	10.1
4	9	6– 16	10.2
5	12	2– 19	10.5
6	6	10– 26	16.8
7	9	7– 23	13.3
8	7	7– 27	14.4
9	5	8– 24	18.2
10	4	10– 30	16.3
11	7	11– 25	19.9
12	5	15– 38	24.2
13	9	10– 36	17.0
14	8	14– 31	20.5
15	5	16– 24	19.4
16– 20	20	16– 44	26.0
21– 25	14	19– 54	29.6
26– 30	10	19– 65	37.0
31– 50	8	22– 80	46.9
51– 90	3	52– 78	67.0
91–119	2	91–119	105.0

$$\frac{\text{Total X-ray}}{\text{Total cracking}} = \frac{3761}{2485} = 1.5 \text{ for all 173 samples.}$$

cracking or flotation or other technique. Thus it may prove to be the most accurate means of measuring internal insect infestation.

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AN EVALUATION OF FIVE PROCEDURES FOR THE DETERMINATION OF INTERNAL INSECT INFESTATION OF WHEAT

VI. INVESTIGATIONS ON THE X-RAY INSPECTION OF WHEAT

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This paper deals with investigations of radiographic techniques as applied to grain. Milner, *et al.* (1, 2) gave data on the X-ray testing of grain. Following publication of their work, the problem was discussed with them, and investigations were extended in an effort to produce a type of radiograph suitable for routine detection of kernels with gross internal insect damage. Material was exchanged between Milner and this laboratory, and equipment differing from that used by Milner was used to determine the best technique for securing suitable radiographs. Variations in energy levels, and the use of filters were investigated as means of producing the proper degrees of contrast on X-ray film. Using infested wheat kernels which had been fastened to a paper, radiographs were made by various techniques. Thus it was possible to evaluate the radiographs by dissecting and examining all of the kernels. At the same time, the 173 radiographs discussed in Part V of this paper were studied in order to evaluate them in terms of two factors: (1) the ability of the radiographic technique to register insect damage on the film and (2) the film quality as it met the requirements of rapid routine examination. (It should be borne in mind that the radiographic image of insect infested wheat reveals both gross and minor damage. As stated in Part V, examinations with the naked eye or low magnification which attempted to assess the total amount of internal damage, no matter how small, were found to be too time consuming. A practical radiographic examination is necessarily limited to gross damage).

Regarding the first factor, the dissection of individual kernels and results on the 173 samples confirmed the work of earlier investigators, viz., that X-ray procedures could be used to measure internal insect infestation of wheat. In some cases, the larva or pupa could be seen on the radiographic image. In other cases, the meaning of doubtful shadows was determined by dissection of the kernel. After the dissection of all suspected kernels, those remaining glued to the mount were also dissected and examined. No internal damage was found in kernels showing no damage on the radiographs.

In appraising the second factor, it was noted that during examination of the 173 radiographs, made using a cobalt-target Machlett AEG-50 X-ray diffraction tube with beryllium window (kernels 75 cm. from the exit

port, Eastman type A film, 15 ma, 13 kv, 30 to 60 seconds exposure) that the high contrast* rapidly produced eye fatigue in the observer. By analogy with ordinary photomicrographic techniques using visible light, it was reasoned that detail would be lost if the radiograph did not exhibit a density gradation relative to the thickness of the subject. In other words, there was little shadow or detail at the thicker portions of the kernel, while the thinner portions were as dense as the background. Conversely, if exposure was increased in order to obtain proper penetration of the thicker portions of the grain, the edges became so severely "undercut" from the scatter of the long wave length, low energy X-rays that they blended with the background, and the kernel appeared smaller than was actually the case.

In an attempt to eliminate these difficulties various techniques were employed. Preliminary studies were carried out on the samples previously radiographed by the technique described above. The mounted specimens were again radiographed with an oil-cooled, portable radiographic head.† The inherent filtration of this type of head (including glass tube, oil, and plastic window) was so great that low energy X-rays were not emitted. The most satisfactory radiographs were produced at 18.5 kv and 10 ma for 3 minutes, using Eastmen type A film. (Type M film required an increased exposure.) Radiographs obtained by this technique showed excellent detail with no observable undercutting, and the absence of intense contrast reduced eye fatigue. With the edges of the kernels clearly defined and the roundness of the kernel visible, the external or internal position of the cutting was more easily interpreted. However, some observers felt that the contrast of these radiographs was too low. The radiographs did demonstrate that satisfactory X-radiation could be produced with a tungsten target tube.

To investigate the factors contributing to the differences in the above radiograph, and in an attempt to produce a radiograph of higher but not excessive contrast without undercutting, a series of radiographs was made with a tungsten target, beryllium window tube. Exposures were made at several voltages; target to film distance was varied, and aluminum filters were used to reduce the amount of "soft" radiation. From the exposures so made the following conclusions were reached (Figure 1):

1. At the lower kv range, comparatively little radiation is emitted from an X-ray head with a glass tube, oil bath, and plastic exit port between the target and film. However, a satisfactory radiograph of comparatively low contrast but with excellent detail can be obtained from such a head at 18 kv and 10 ma.

2. A cobalt target, beryllium window tube produces a very high contrast picture with accompanying loss of detail at the edges of the kernel

* With high contrast the image of the grain is comparatively clear with an abrupt gradation to a black at the edge of the kernel which merges with the black background.

† Equipment loaned by X-Ray Division, Westinghouse Electric Corporation, Baltimore, Maryland.

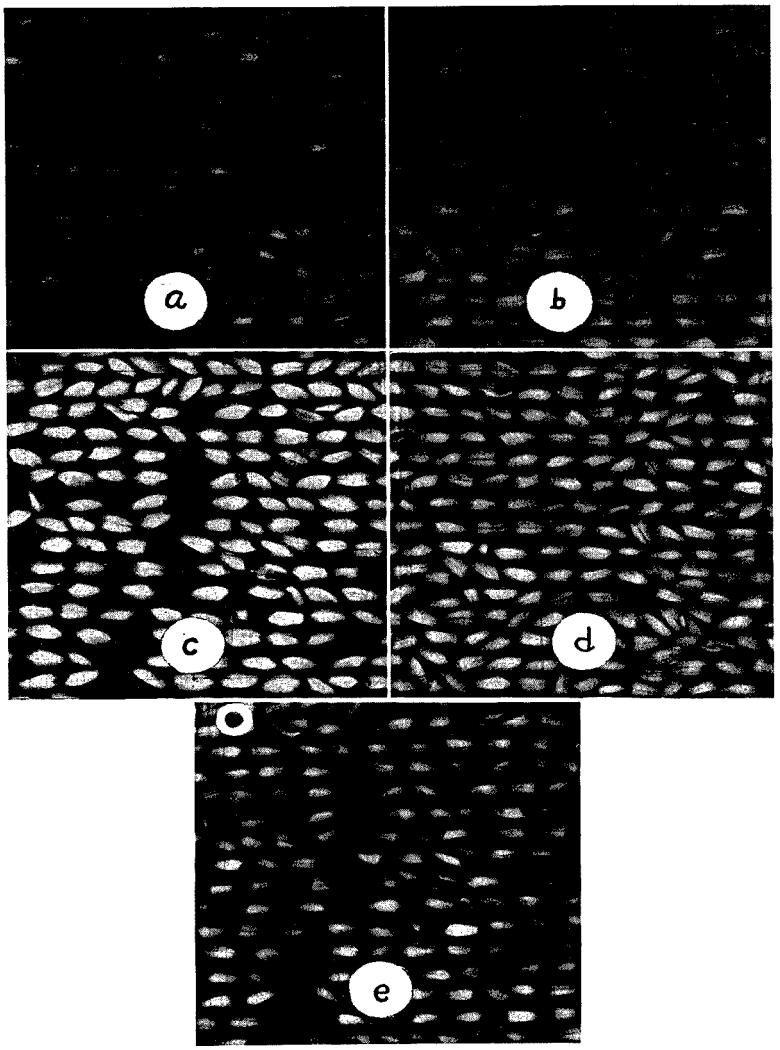

FIG. 1.—Radiographs of Wheat.

a. High contrast made with a cobalt target beryllium window tube at 13 kv.
b. Low contrast made with a tungsten target glass tube in oil bath and with plastic window to the head at 18.5 kv.
c. Fairly high contrast made with a tungsten target beryllium window tube at 15.5 kv. 26 inches.
d. Fairly low contrast made as in C except with 0.2 mm aluminum as a filter.
e. High contrast made as in C at 11 inches.

and a lack of penetration at the center. However, with intense illumination these radiographs will show gross insect damage.

3. The radiation emitted by a tungsten target, beryllium window tube contains more "hard" X-rays than the cobalt target tube. This results in increased penetration without overexposure of the background and thinner parts of the kernel.

4. The use of aluminum filters over the exit port eliminates some of the "soft" X-rays from the beryllium window tube. The use of 0.2 mm of aluminum improves the quality of radiographs made at 15.5 kv, 10 ma and 26 inches. Less than 0.2 mm has little effect on the quality of the radiographs, and 0.5 mm may be preferred by some workers. (Compare "c" and "d," Figure 1.)

5. There is an appreciable difference between radiographs made at 11 and at 26 inches from the target. (The 26-inch column of air absorbs some of the longer X-rays and thus reduces film contrast. Compare "c" and "e," Figure 1.)

6. The use of two Wratten filters (Nos. 29 and 58) has been suggested (3) for the window in the film holder. Such material does have uniformly high X-ray transmission properties and is desirable when using a beryllium window, but it is expensive and relatively fragile. Since this work was started, X-ray film casettes with a light-opaque plastic window having uniformly high X-ray transmission have been made commercially available.

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BOOK REVIEW

Introductory Mycology. By CONSTANTINE JOHN ALEXOPOULOS, John Wiley and Sons Inc., 440 Fourth Avenue, New York 16, New York. 1952. xiii+482 pp. $7.00.

This comprehensive text is the outgrowth of a need for a textbook presenting data about the fungi in such a manner that the student who has no grounding in the subject matter will be able to grasp more easily the fundamentals of the "structure and classification." The object of the book is to eliminate, as much as possible, the detailed data which are intended primarily for the "specialist."

The text contains seventeen chapters dealing with as many classifications of the various forms of organisms. The opening chapter is an excellent description of "The Fungi." It gives a clear concept of what are termed fungi and their importance and relationship to man. It also includes general characteristics, reproduction processes, explaining both sexual and asexual phases, and the classification of the fungi. The author attempts to make his system of classification as simple as possible and at the same time include in it the most recent data pertaining to classification. In this connection he has adopted the more recent idea of the taxonomists in that he divides the division of the plant kingdom formerly called the "Thallophyta," into three "Phyla." These "Phyla" he designates as the "Bacteria (Schizomycophyta)," the "Slime Molds (Myxomycophyta)," and the "Fungi (Eumycophyta)." The author does not consider this system as the only form to be followed, as he believes any system is subject to change when new data concerning the structure of fungi are found. In some parts of the text he follows the older systems of classification.

The next fifteen chapters deal with the forms of organisms classified under each of the three "Phyla" described above. Each of these chapters include some general introductory remarks, something of the occurrence and importance of the organisms to man and some general characteristics of the whole group. The classification of the organisms in each particular group is included. There is also included a typical life cycle of some representative form.

The final and seventeenth chapter deals with a short treatise on "The Lichens." The author gives a general description of these forms and states that the present system of classifying these forms is as satisfactory as any and that any change would only cause further confusion.

At the end of each chapter there is presented a summary or resume of the facts in that chapter. This rounds up the main features and serves as a good source of review. Following this summary the author gives a list of references pertaining to the subject matter just covered. This is an excellent feature in that it furnishes the student with material for further investigation while the subject matter is fresh in his mind. Very few of the references are taken from foreign literature so that the student is not confronted with the necessity of attempting to translate an article in some foreign language.

The author has added a new feature in introducing the student to the numerous mycological terms that are found in this field of study. As each of these terms are used, the author gives either the Greek, Latin, or other derivation of the term with its meaning, so that the student is given a definition of the term when used and does not have to consult a glossary for its meaning. In addition there is also an excellent glossary at the end of the book.

The text is well illustrated with both drawings and photographs. There are numerous diagramatic drawings of life cycles of the various forms which clearly explain the sexual and asexual phases.

The book is well organized and incorporates the most recent data and theories pertaining to the study of mycology. It is written in a clear, concise manner which makes it readily applicable as a text on "Introductory Mycology."

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